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In vitro and *in vivo* acaricidal activity and residual toxicity of spinosad to the poultry red mite, *Dermanyssus gallinae*

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ABSTRACT

This paper describes two experiments conducted to examine the acaricidal potential of spinosad against the poultry red mite, *Dermanyssus gallinae* (De Geer), a serious ectoparasitic pest of laying hens. Spinosad is a natural product derived from the fermentation of the micro-organism *Saccharopolyspora spinosa. In vitro* testing confirmed that, when applied to a galvanised metal plate to the point of run-off, spinosad was toxic to adult female *D. gallinae* and suggested that at an application rate of 3.88 g/L a significant residual toxicity of spinosad could be achieved for up to 21 days. A subsequent *in vivo* experiment in a conventional cage housing system for laying hens demonstrated the acaricidal activity and residual toxicity to *D. gallinae* of a single application of spinosad when applied at either 1.94 or 3.88 g/L. Residual toxicity of spinosad at both of these application rates was maintained throughout the course of the 28 day post-spray study period, with a peak in product efficacy seen 14 days after spraying. The results suggest that the greater the *D. gallinae* population the greater will be the toxic effect of spinosad. Although the exact reasons for this are unclear, it can be speculated that conspecifics spread the product between each other more efficiently at higher mite population densities. However, further study is warranted to confirm this possibility. Application of spinosad *in vivo* had no effect on hen bodyweight or egg production parameters (number and weight), suggesting that this product could be used to effectively control *D. gallinae* infestations whilst birds are in lay. This paper also describes a novel method for effectively and efficiently achieving replication of treatments in a single poultry house, previously unpopulated with *D. gallinae*. Individual groups of conventional cages were stocked with hens, seeded with *D. gallinae* and used as replicates. Independence of replicates was achieved by isolating cage groups from one another using a non-drying glue barrier to minimise *D. gallinae* migration. Creating isolated populations (replicates) of *D. gallinae* within a single poultry house thus represents a novel and efficient means of screening other potential acaricides under field conditions.

1. Introduction

The poultry red mite, *Dermanyssus gallinae* (De Geer), is currently the most economically deleterious ectoparasite of laying hens in Europe (Chauve, 1998). Worldwide, *D. gallinae* prevalence in laying flocks varies from 20% to 90%, depending upon the country and production system considered (Sparagano et al., 2009). In the UK, infestation levels of between 60% (Fiddes et al., 2005) and 85% (Guy et al., 2004) can be expected in commercial egg laying premises, with higher mite populations typically seen in free-range systems compared to cage units (Guy et al., 2004; Fiddes

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et al., 2005; Arkle et al., 2006). This is of particular concern given that conventional cages will be prohibited in the EU from 2012 (EU Directive 99/74/EC) and thus the proportion of hens housed in alternative systems such as free-range is likely to increase substantially.

Where infestations of *D. gallinae* are sufficiently severe, mite feeding may result in significant stress to hens with a resulting negative impact on bird condition, growth rate, egg quality (through increased shell thinning and spotting) and egg production (Urquhart et al., 1996; Chauve, 1998). In extreme cases, *D. gallinae* population levels may be so high that anaemia, and even death of hens, can result from infestation (Wojcik et al., 2000; Cosoroaba, 2001). Whilst low level mite infestation may pose fewer problems to the host birds, it has been reported that *D. gallinae* may serve as a vector for numerous poultry pathogens (Chirico et al., 2003; Valiente Moro et al., 2009). This suggests that even small numbers of *D. gallinae* in poultry systems may have a serious impact upon the production and welfare of laying hens.

Economic costs associated with *D. gallinae* due to both control and reduced production have been estimated at €130 million annually for the EU egg industry (van Emous, 2005). Control of *D. gallinae* has typically been achieved by the use of synthetic contact acaricides including carbaryl, diazinon, dichlorvos and permethrin, but the continued use of these products is hampered by issues of mite resistance (Beugnet et al., 1997; Kim et al., 2004, Fiddes et al., 2005) and decreasing product availability as a consequence of more stringent legislation.

Spinosad was first registered for agricultural/horticultural use in the late 1990s and by 1999 it was approved for use on over 100 crops in 24 different countries (Thompson et al., 2000). Being a natural product derived from fermentation of the micro-organism *Saccharopolyspora spinosa* (Mertz and Yao), spinosad possesses several favourable characteristics for a pesticide. For example, Anastas et al. (1999) report that spinosad will not bio-accumulate, volatize or persist in the environment and will degrade naturally when exposed to light. In addition, spinosad has been found to display activity against a range of insect pests, especially those in the genera Lepidoptera, Diptera and Thysanoptera, and to a lesser extent the Coleoptera and Orthoptera (Thompson et al., 2000). Corresponding research has also found that spinosad possesses relatively low toxicity to mammals and birds, where acute oral LD_{50} values of >5000 and >2000 mg/kg have been reported for mice and mallard ducks, respectively (Thompson et al., 2000). Research also suggests that 70–90% of beneficial insects are left relatively unharmed by spinosad (Anastas et al., 1999), where for certain ladybirds, lacewings and predatory mites, LD_{50} values are >1000× greater than with cypermethrin (Thompson et al., 2000). The toxicity of spinosad to mites *per se* has been reported as being variable and/or reduced in comparison to insect species (Thompson et al., 2000; Villanueva and Walgenbach, 2006; Holt et al., 2006). Experiments using spinosad against the ectoparasitic cattle tick *Boophilus microplus* have nevertheless yielded promising results (Davey et al., 2001, 2005), as has work with other soft and hard tick species (Cetin et al., 2009). It is therefore possible that spinosad could provide a new and effective control product for use against *D. gallinae*, whilst fulfilling additional desirable environmental and non-target organism toxicity criteria.

Therefore, the aim of this study was to test the acaricidal activity of spinosad to *D. gallinae* at different concentrations and varying times after product application. This was done initially using an *in vitro* design in a laboratory experiment, followed by an *in vivo* experiment to determine the effectiveness of spinosad against *D. gallinae* under conditions which mimicked commercial egg production.

2. Materials and methods

2.1. In vitro experiment

2.1.1. Experimental design and treatments

The experiment was designed as a four × eight factorial, with four application rates of spinosad and eight time points post-spraying (PS) giving 32 exposure treatments in total. Spinosad solutions were made using the commercial product Elector® (Elanco Animal Health, Basingstoke; 452.8 g/L spinosad) diluted in distilled water to give concentrations of 0.00, 0.97, 1.94 and 3.88 g/L spinosad. Solutions were applied to the test surfaces as a coarse spray using a standard 500 ml hand-operated atomiser. Surfaces were sprayed to the point of run-off and allowed to air dry. For each application rate of spinosad, *D. gallinae* were exposed to the treated surfaces at one of eight time points PS; 2.5 h, 1, 3, 7, 10, 14, 21 and 28 days. There were four replicate test units per treatment, and the study was implemented by a technician who remained blinded to the experimental treatments.

2.1.2. Source of D. gallinae and test apparatus

D. gallinae were collected from a free-range laying unit in Northumberland (England), brought to the laboratory at Newcastle University (England) and stored for a period of 48 h prior to use. Recently fed adult female mites were then placed in the test unit, namely a galvanised metal plate (70 mm × 70 mm × 3 mm; Metal Supermarket, Gateshead, England) which had been previously coated with one of the four different application rates of spinosad. Mites were contained on the metal plate by an inverted Petri dish, coated with the same concentration of spinosad. A Vaseline seal was used around the Petri dish base to ensure that mites could not leave the test arena. The metal plates and Petri dishes were contained within a fume cupboard prior to the addition of *D. gallinae*, where conditions of continuous air-flow were maintained throughout the study in an attempt to mimic conditions in a ventilated poultry house. Once mites were placed into the test unit, they were transferred to a climate-controlled growth room maintained at 22 °C with a 16:8 light:dark cycle.

Approximately 25 *D. gallinae* were used per test unit. The proportion of live (active, clear locomotion), moribund (passive, hardly any locomotion, but showing some movement, e.g. of legs) and dead *D. gallinae* (no movement) was calculated after exposure to the treatments for 48 h