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Primary screen for potential sheep scab control agents

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ABSTRACT

The efficacy of potential acaricidal agents were assessed against the sheep scab mite *Psoroptes ovis* using a series of *in vitro* assays in modified test arenas designed initially to maintain *P. ovis* off-host. The mortality effects of 45 control agents, including essential oils, detergents, desiccants, growth regulators, lipid synthesis inhibitors, nerve action/energy metabolism disruptors and ecdysteroids were assessed against adults and nymphs. The most effective candidates were the desiccants (diatomaceous earth, nanoclay and sorex), the growth regulators (buprofezin, hexythiazox and teflubenzuron), the lipid synthesis inhibitors (spirodiclofen, spirotetramat and spiromesifen) and the nerve action and energy metabolism inhibitors (fenpyroximate, spinosad, tolfenpyrad, and chlorantraniliprole).

1. Introduction

Sheep scab is a serious animal welfare condition caused by the skin parasite *Psoroptes ovis* (Acari: Psoroptidae) which causes an allergic dermatitis through faecal deposition on the skin of the animal (Sinclair and Kirkwood, 1983; Good, 1996; Lewis, 1997). The acuteness of reaction to the presence of the parasite, which ranges from mild to severe, is dependent upon breed and pre-exposure to mites (Fourie et al., 1997; Bates, 2000). Transmission of the disease occurs primarily from sheep-to-sheep contact but can also occur through contact with infected areas in the environment such as housing, fencing or farm equipment. Although *P. ovis* are obligate ectoparasites, they are capable of surviving off the host for periods of time (O'Brien et al., 1994). The disease is highly contagious and quickly spreads throughout the flock (Sargison et al., 1995). Infected animals will tend to scratch large areas of their fleece resulting in extensive bald patches. Careful inspection of the affected skin lesions reveals the presence of mites (Lewis, 1997). Once the disease becomes established, the animals experience weight loss leading to poor general condition, debilitation and the birth of weak lambs which have associated higher mortality rates than non-infected animals (Sargison et al., 2006).

Having been eradicated from the UK in 1952, before being accidentally reintroduced in 1973, the disease is now endemic throughout the country (Sargison et al., 2007). Deregulation allowed farmers to choose their own treatment regime (Smith et al., 2001). Previous studies estimate that only 40% of farmers treat their sheep prophylactically against scab in spite of the high animal welfare costs and the associated economic costs of the disease (Milne et al., 2007; Bisdorff and Wall, 2008). As a result, since deregulation, sheep scab has become increasingly widespread throughout the UK (Milne et al., 2007). Additional control and eradication of the disease in the UK is now considered essential by some (Sargison et al., 2006; Rose et al., 2009). In 2005, sheep scab was thought to cost the British sheep industry in the region of £8 million per annum (Nieuwhof and Bishop, 2005) and current feedback from the industry suggests that the incidence of sheep scab is increasing, with regulatory withdrawals, resistance to current pesticide treatments and unavailability of suitable replacements being key issues (SCOPS, 2005; Lewis, 2013). There are also serious concerns about risks posed to both operators and the environment by many conventional pesticides.

The Insecticide Resistance Action Committee (http://www.irac-online.org) defines at least twenty seven different modes of action (MoA) for insecticides/acaricides of which many compounds or type compounds within the classification have not been tested fully against mites, and very few against *P. ovis*. Many of these classes of compound are either developmental or feeding disruptors, or have other non-neurotoxic MoA. Many are also considered to be potentially more environmentally benign and safer than the rapidly acting acetylcholine esterase inhibitors such as the organophosphate diazinon used in sheep dips. They may also offer realistic alternatives to the macrocyclic lactones. This paper reports on the results of a primary screen of over 40 chemicals from seven different classes using a modified *in vitro* method designed for maintaining *P. ovis* off-host (Thind and Ford, 2007).

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2. Materials and methods

2.1. Mites

Psoroptes ovis were collected from the pelts of experimentally infested adult ewe sheep. The mites were examined under a binocular microscope at magnification of 10–50× and grouped according to sex and stage following the morphological descriptions of Sanders et al. (2000). The groups required for these experiments consisted of adult females and nymphs (protonymphs).

2.2. Chemicals

For analysis purposes, the chemicals were grouped into seven categories: essential oils, desiccants, detergents, growth regulators, lipid synthesis inhibitors, nerve action and energy metabolism inhibitors and ecdysteroids. Not all the chemicals were tested against both the adults and nymphs, as mite numbers at the time of testing were sometimes limiting. The concentration ranges were based on potential practical economic costs for the end-user, toxicity and eco-toxicity implications.

2.2.1. Essential oils

Menthol (99%, Sigma-Aldrich, M277-2), carvacrol (98%, SAFC, Sigma-Aldrich, W224502), geraniol (98%, Sigma-Aldrich, 163333), cinnamaldehyde (98%, SAFC, Sigma-Aldrich, W228605), eugenol (99%, Alfa Aesar, Heysham U.K., LO3559), thymol (98%, Alfa Aesar, LO4966), L-menthol (99%, Alfa Aesar, LO3829), limonene (97%, Alfa Aesar, LO4733), citronellol (95%, Alfa Aesar, LO5745), citronellal (96%, Alfa Aesar, L15753), terpinen-4-ol (97%, Acros Organics, 360020250), garlic oil (98%, Sigma-Aldrich, W530316).

2.2.2. Desiccants

Sorex (sorbitol silica powder, Sorex Ltd., Widnes, U.K.), diatomaceous earth (DE) (Silico-sec®, Biofa AG, Münsingen, Germany), nanoclay (Sigma-Aldrich, 685445).

2.2.3. Detergents

Tween 20 (Sigma-Aldrich, P-1379), triton (Fluka, Sigma-Aldrich, x-100), nonidet (Sigma-Aldrich, 74385), diethylaminobenzaldehyde (DEAB; Sigma-Aldrich, D86256), sodium dodecyl sulphate (SDS; Sigma-Aldrich, L4509), amprolium hydrochloride (AHCl; Sigma-Aldrich, A0542), ethylhexadecyldimethylammoniumbromide (EHDEAB; Sigma-Aldrich, C0636) hexadecyltrimethylammonium-*p*-toluenesulfonate (HTA-P-T; Sigma-Aldrich, C8147).

2.2.4. Growth regulators

Methoprene (98%, 33375), hydroprene (99%, 46426); clofentezine (99.9%, 36763), hexythiazox (99.9%, 33365); chlorfluazuron (97.8%, 36530), flufenoxuron (98.3%, 46069), hexaflumuron (99.2%, 37902), teflubenzuron (98.8%, 45756); buprofezin (99.2%, 37886); tebufenozide (99.2%, 31652), all obtained from Fluka, Sigma-Aldrich.

2.2.5. Nerve action/energy metabolism disruptors

Spinosad (98%, Fluka, Sigma-Aldrich, 33706); imidacloprid (98%, Fluka, Sigma-Aldrich, 37894); milbemycine oxime (97%, Novartis, technical); fenpyroximate (99%, Fluka, Sigma-Aldrich, 31684), tolfenpyrad (99.2%, Fluka, Sigma-Aldrich, 37043); chlorantraniliprole (98.1%, Fluka, Sigma-Aldrich, 32510).

2.2.6. Lipid synthesis inhibitors

Spirotetramat (99.5%, Fluka, Sigma-Aldrich, 32713), spiromesifen (99.8%, Fluka, Sigma-Aldrich, 33599), spirodiclofen (99.3%, Fluka, Sigma-Aldrich, 33654).

2.2.7. Ecdysteroids

20-Hydroxyecdysone (20-HE, 93%, Fluka, Sigma-Aldrich, H5142), ecdysone (97%, Sigma-Aldrich, E9004), ponasterone (65%, Sigma-Aldrich, P3490).

2.3. Preparation of test compounds and their application

For all compounds, other than the desiccants, the base filter papers (Whatman No. 1, 42.5 mm diameter) of the test arenas were impregnated with lipid fraction extracted from sheep pelt (mainly cholesterol, followed by lesser amounts of other sterols and waxes) using chloroform prior to pesticide treatment. Previous studies (J. A. Dunn, J.C. Prickett, B. Thind; unpublished observations) have shown that addition of this extract increases off-host survival and improves *in vitro* oviposition and moulting of *P. ovis*.

Sheep serum was provided as a food source for the mites during the bioassays, and was obtained by allowing whole sheep's blood to coagulate and then carefully decanting the resultant supernatant. The supernatant was then centrifuged at 12,000*g* for 10 min at 8–12 °C (Sorval RC5C, Thermo Fisher Scientific Inc.).

Stock solutions of each test chemical were prepared by diluting a known quantity with acetone. For each acaricide, aliquots of the stock solution (20 g/L) were further diluted with acetone to provide the relevant concentrations. Using a 1 ml pipette, a 0.2 ml aliquot of the appropriate concentration of the acaricide was spread evenly on each filter paper. A spiral motion of progressively decreasing radius was used to ensure an even distribution of the acaricide on the filter papers.

Impregnation of the filter papers was carried out in ascending order of pesticide concentration. In addition, filter papers were treated with acetone plus extract only to act as controls. Treated and control papers were placed on pin boards for at least one hour so that the diluent could evaporate thoroughly.

The desiccants were applied as a dry dust. The required amount of desiccant was placed in the centre of a plastic Petri dish (base, 53 mm diameter, 12 mm high; top 56 mm, 7 mm high). The Petri dishes were joined, top to top, or base to base with a strip of Parafilm®. The dishes were then shaken and tapped to distribute the dust evenly between both dishes. Static electricity caused the desiccants to adhere to the surfaces.

2.4. Exposure of P. ovis to treated papers and dishes

The treated and control filter papers were incorporated into modified arenas described by Thind and Ford (2007) (Fig. 1). Briefly this consisted of three glass sheets (68 mm × 68 mm × 4 mm) each with a 35 mm diameter central hole. The two outer sheets had a moulded 42.5 mm diameter filter paper (Whatman No. 1, pressed into a dish shape) incorporated to make a chamber (about 8 mm deep) and a thin glass sheet (68 mm × 68 mm × 1 mm) added to make a base. A sterile pad (Steropad, Steroplast, 3008155), was cut into 1 cm^2 (0.03–0.035 g). One of these squares was placed on the centre of this glass sheet and 0.6 ml of serum added, this was sufficient for the liquid to just seep through onto the lower filter paper. The arena was held together using two 'bulldog' paper clips (Premier Grip PG1900).

For the adult assays, 25 freshly harvested female *P. ovis* were placed on the base filter paper in the test arenas. Five replicates were prepared for each dose and control. For the experiments with the nymphs, only one protonymph was placed in each arena, with fifteen replicates for each dose and control. The arenas were then