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By Trevor A Lambkin

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Researcher Contact Details

Trevor A Lambkin
Agri-Science Queensland
Department of Employment, Economic Development and Innovation
Locked Mail Bag No 4
MOOROOKA QLD 4105

Phone: 07 3362 9606
Fax: 07 3362 9631
Email: trevor.lambkin@deedi.qld.gov.au

In submitting this report, the researcher has agreed to RIRDC publishing this material in its edited form.

RIRDC Contact Details

Rural Industries Research and Development Corporation
Level 2, 15 National Circuit
BARTON ACT 2600

PO Box 4776
KINGSTON ACT 2604

Phone: 02 6271 4100
Fax: 02 6271 4199
Email: rirdc@rirdc.gov.au.
Web: http://www.rirdc.gov.au

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or phone 1300 634 313

Foreword

For approximately three decades the Australian broiler industry has relied heavily on the use of insecticides as its key tool for management of darkling beetle or lesser mealworm, *Alphitobius diaperinus* [Panzer] in broiler houses.

The use of these chemicals over this period has been largely unchecked which has resulted in the development of strong insecticide resistance in many beetle populations from broiler farms. Although we are in a period now with an improved knowledge of managing resistance and the availability of new more effective insecticides that are currently marketed, the industry still requires more pest management options in order to inhibit development of resistance and reduce overall chemical use.

In response to this need, 'natural' agents such as entomopathogenic nematodes and fungi were proposed as potential agents for managing darkling beetle populations in Australian broiler houses. Since 2007 laboratory and field studies have been undertaken to assess these agents.

This report outlines these studies and discusses potential benefits to the Chicken Meat industry resulting from this research. This project was funded from industry revenue, which is matched by funds provided by the Australian Government.

This report is an addition to RIRDC's diverse range of over 2000 research publications and it forms part of our Chicken Meat R&D program, which aims to support increased sustainability and profitability through focused research and development.

Most of RIRDC's publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
Rural Industries Research and Development Corporation

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What the report is about

This report describes the trialling of biological agents for the management of lesser mealworm in broiler houses.

Who is the report targeted at?

1. The Chicken Meat Research Program of RIRDC;
2. The Australian Chicken Meat Industry and
3. Chicken meat producers.

Background

Lesser mealworm or darkling beetle, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) are common insect pests of broiler houses throughout the world. As they can function as vectors for a large number of avian diseases and parasites and can carry food borne diseases, large lesser mealworm populations pose significant threats to broiler flock health and the production of safe food. They are also structural pests of broiler houses, causing damage to compacted earth floors, and ceiling and wall insulation. In addition, the quality of nutrition for broiler chickens can be significantly compromised by birds consuming large numbers of lesser mealworm larvae and adults. The application of residual insecticides to the floors and lower walls of broiler houses is the standard management method for lesser mealworm in Australia. Currently mostly three insecticides are used; fenitrothion, since the 1970s, cyfluthrin, since about 1995, and spinosad, which gained registration for broiler house use in early 2007. Recent studies confirmed that widespread and often high levels of resistance to fenitrothion and cyfluthrin occur in lesser mealworm broiler house populations in eastern Australia. Because of the inadequacies of long-standing control practices and the prevalence of insecticide resistance, novel agents for the management of lesser mealworm were assessed. Results of previous entomopathogenic nematode laboratory work indicated that *Steinernema carpocapsae* (an off-the-shelf-product) was the most viable and effective nematode species when applied to bedding, for temperatures around 30ºC. For entomopathogenic fungal work, *Beauveria bassiana* was found to be a more effective bedding treatment for lesser mealworm than *Metarhizium anisopliae*. This information was then used to devise field trial protocols to test the efficacy of entomopathogenic nematodes and fungi for management of lesser mealworm in broiler houses.

Aims/objectives

In light of the need to manage and reduce insecticides across all animal industries, the project aimed to trial natural biological agents in the field and laboratory, singly and in combination with other agents, as bedding and earth treatments for the management of lesser mealworm in broiler houses. In addition it tests the compatibility of these agents with three disinfectants frequently used by the broiler industry.

Methods used

This project entailed laboratory testing the effect on virulence of entomopathogenic nematodes (*Steinernema carpocapsae*) and fungi (*Beauveria bassiana* and *Metarhizium anisopliae)* to lesser mealworm when exposed to three broiler house disinfectants; a whole broiler house trial comparing the efficacy of entomopathogenic nematodes to spinosad in reducing numbers of lesser mealworms; and small plot trials comparing the efficacy of the above three biological agents (including some in combination with spinosad and diatomaceous earth) to spinosad and cyfluthrin in reducing lesser mealworms.

Results/key findings

The results from the laboratory trials which assessed the effect of disinfectants on the virulence of entomopathogenic nematodes and fungi indicated that almost all treatments of Protosan DS® and Virkon S® tested (including field application rates) had no significant effect on the three agents' virulence even when applied concurrently with disinfectants. The large scale applications of entomopathogenic nematodes to broiler houses trialled in this study overall gave disappointing results. This was in contrast to the very positive data that came from previous laboratory studies. Apart from one house which received a narrow band nematode application under the feed supply lines and the two spinosad treated houses almost all other houses (control and nematode treated) saw increases in total beetle numbers from the first to the second batch. Trials using treated small plots under feed pans in general gave inconclusive results that were mostly inconsistent and did not mirror the results of comparable laboratory tests. In essence, the measurement of the effectiveness of discrete applications of control agents applied under feed pans can be significantly compromised by the active movement of lesser mealworm larvae, mostly travelling to under feed pan areas.

Implications for relevant stakeholders

The trialling of biological agents unfortunately has provided no conclusive results to base action on. In addition to this, results indicated that two of the three disinfectants had no measureable effect on the efficacy or mortality of the entomopathogenic fungal and nematode species tested.

Recommendations

There is no use in further testing diatomaceous earth and entomopathic fungi and nematodes as beetle control agents.

1. Introduction

The lesser mealworm or darkling beetle, *Alphitobius diaperinus* (Panzer) is a common cosmopolitan insect pest of broiler houses, predominately those with earth floors, wherein it occurs in large numbers in the bedding used on the floors of the houses. Economic losses arising from infestations are related to the insect's competency as a reservoir of avian disease agents and parasites and its ability to destroy compacted earth floors and insulation materials within broiler houses. In addition, the pest is known to transmit food-borne diseases such as rotavirus, *Escherichia coli* and *Salmonella enterica* (serovar typhimurium), and has been implicated in the transmission of *Campylobacter* spp. The application of residual insecticides to the floors and lower walls of broiler houses is the standard management method for lesser mealworm in Australia. Currently three insecticides are mostly used; fenitrothion, since the 1970s, cyfluthrin, since about 1995, and spinosad, which gained registration for broiler house use in early 2007. Recent studies confirmed that widespread and often high levels of resistance to fenitrothion and cyfluthrin occur in lesser mealworm broiler populations in eastern Australia. Because of the inadequacies of long-standing control practices and the prevalence of insecticide resistance, novel agents for the management of lesser mealworm are required.

In a previous RIRDC funded project the susceptibility testing of lesser mealworm to four species of entomopathogenic nematode and to two species of entomopathogenic fungus was completed. Results of this nematode work indicated that *Steinernema carpocapsae* (an off-the-shelf-product) was the most viable and effective nematode species when applied to bedding for temperatures around 30ºC. For the fungal work, *Beauveria bassiana* was found to be a more effective bedding treatment for lesser mealworm than *Metarhizium anisopliae*. The information gleaned from this preliminary research was then used to devise field trial protocols to test the efficacy of entomopathogenic nematodes and fungi for the management of lesser mealworm in broiler houses.

2. Objectives

Previous investigations into the management of lesser mealworm (darkling beetle) in Australian broiler houses has shown that high pest numbers predominately occur in broiler house litter, and the treatments of the compacted earth floors of broiler houses with two of the currently registered insecticides, viz. fenitrothion and cyfluthrin appear to have little effect in reducing pest numbers. This research also indicated that control agents seem more effective when applied to fresh litter than to earth floors. In light of the need to also reduce insecticide use across all animal industries, the current project aimed at trialling natural bedding and earth treatments for the management of lesser mealworm in broiler houses. Specifically, to complete final laboratory assays to determine a suitable application regime for entomopathogenic fungi and nematodes in broiler houses, including an assessment of the agents' compatability with broiler house disinfectants; and to undertake small plot field trials to measure fungal and nematode efficacy in reducing darkling beetle populations in broiler houses.

3. Methodology

Large scale field trialling of entomopathogenic nematodes (*Steinernema carpocapsae*)

Eight broiler houses on a broiler farm near Gatton were used for the field study. Each broiler house measured 125 m by 15m (i.e. 1875 m^2); each house with three feed supply lines (each line approximately 122 m long). All houses were tunnel-ventilated with earth-floors, with House 4 having a thin cement/sand based floor (Weslig®). The bedding litter was completely removed from each house at the end of each batch (broiler flock time) and fresh bedding (locally milled wood shavings) was spread onto the floors. The standard insecticide treatment regime for the farm was floor applications of Elector PSP® (spinosad). The study was conducted over two batches; for the first batch, the floors of all houses received a treatment of spinosad. For the subsequent batch, four houses received floor applications of entomopathogenic nematodes under feed supply lines, two houses received floor applications of spinosad under feed supply lines, while another two houses were left untreated. All treatments were randomly assigned. Applications of spinosad were sprayed as water-based suspensions using nozzle applicators, while nematodes were applied with the same nozzle applicators but using a much greater volume of water than spinosad. Nematode application rates were adapted from previous laboratory studies (as per RIRDC project report DAQ-330A). Town water with a pH 8 was used for all broiler house applications.

Details of the treatments of each broiler house follow.

Batch 1 (commenced 15 April 2008)

All eight broiler houses received whole floor treatment of a label rate spinosad application using 200 mL of Elector PSP® (480 g/L) applied in 50-60 L of water per house.

Batch 2 (commenced 19 June 2008)

House 1: Spinosad Treatment (replicate A), 1 m wide treatments under each of three feed supply lines using total volumes of 78 mL of Elector PSP (480 g/L) applied in 12 L of water, with a treatment under each line using 26 mL of product in 4L of water (i.e. a rate of 0.21 mL of Elector PSP® in 33 mL of water/m^2);

House 2: Control (replicate A), with no water treatment;

House 3: Spinosad Treatment (replicate B): as for treatment in House1;

House 4: Control (replicate B), as for treatment in House 2;

House 5: Nematode Treatment 1 (replicate A), 1m wide treatments under each of three feed supply lines at an application rate of 3.875×10^6 nematodes in 1 L of water/m^2 of earth floor (i.e. 366 m^2) using a total of 1.42×10^9 nematodes (1432 g);

House 6: Nematode Treatment 2 (replicate B), 2 m wide treatments under each of three feed supply lines at an application rate of 3.875×10^6 nematodes in 1L of water/m^2 of earth floor (i.e. 732 m^2) using a total of 2.84×10^9 nematodes (2866 g)

House 7: Nematode Treatment 2 (replicate B): as for treatment in House 6;

House 8: Nematode treatment 1 (replicate B): as for treatment in House 5.

Nematode doses for the four broiler houses (i.e. nematodes applied per m^2) were based on an average number of 9.9×10^5 nematodes/g of nematode formulation. Samples of litter were collected weekly from each house during the time of the field study. Four litter samples, each of 62.5 mL were each collected from under four defined feed pans on the central feed line in the middle area of the brooder section of each broiler house. These samples were collected by plunging a plastic scoop into the litter until the floor of the house was reached, and then a level scoop of litter was removed. In total, 32 litter samples were collected from the farm per week over the time of the field study (i.e. 7-8 weekly samples per batch). Litter samples were transported to the laboratory in vented plastic boxes where live larvae, pupae and adults of *A. diaperinus* were extracted from each sample, counted and discarded.

Effect on virulence of entomopathogenic nematodes (*Steinernema carpocapsae*) to lesser mealworm when exposed to three broiler house disinfectants

The entomopathogenic nematodes used for the testing were derived from larvae of wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) specifically cultured in the laboratory to maintain colonies of nematodes. Broiler house disinfectants tested were Virkon S®, Protosan DS® and formalin. Laboratory test boxes were set up that were analogous to a field situation, i.e. with a compacted earth floor (where disinfectant is normally applied) and a layer of bedding (wood shavings) above the sand, on which the nematodes were applied. Consequently three separate assays were undertaken with the three disinfectants tested per assay (with three replicates of all treatments), in which 18 plastic test boxes (round tapering food containers; 50mm high; bottom radius 42.4 mm, top radius 55 mm: 280 mL) were set up for each assay. In each test box there was a compacted layer of sand (50 g) on the bottom, covered with a layer of wood shavings (6 g), and finally a layer of culture medium (1 g) was added to assist the intrinsic survival of the lesser mealworms.

In each assay (Table 1), the field application rates[1] for the three disinfectants (i.e. g product/m^2) were tested for their compatibility with nematodes and the subsequent effect on virulence of the nematodes to lesser mealworms. In each test box, the amount of disinfectant per surface area of the sand (0.0057 m^2), based on the /m^2 field application rate, was applied in 2.5mL of H_2O. These application rates were: Protosan DS®-6.8×10^{-3}mL of product/2.5 mL of H_2O, Virkon S®-1.5×10^{-2}g of product/2.5 mL of H_2O, formalin- 1.1×10^{-1}mL of product/2.5 mL of H_2O. This was followed immediately by a nematode dose (nematodes/mL/m^2) which was derived from the previous RIRDC project (DAQ-330A). Nematodes were taken from the nematode culture and applied in 2.5 mL of H_2O to the bedding layer (0.0075 m^2). Stock nematode concentrations used for the three separate assays were 5950, 4925 and 3090 nematodes/2.5 mL. To assist the nematode's basic survival in the test boxes, a larger volume of water/m^2 than the prescribed field rate was used for disinfectant applications. Ten mature lesser mealworm larvae were then added with the culture medium to each test box. Control treatments included test boxes treated with just nematodes and also included two negative controls, viz. water and disinfectant only treatments. All treatments, including controls were replicated three times for each assay. Test boxes were incubated at 30°C and ambient relative humidity for 48 hr, after which larvae were assessed for nematode induced mortality.

Differences in mortality between treatments were tested by fitting a generalized linear model to the mortalities for each treatment by assay combination, with terms treatment and assay, binomial distribution and logit link. Pair-wise differences were tested for significance using t-tests on the logit scale. For this analysis the results for the two treatments without nematodes (water only and disinfectant only) were combined as there was only one larval death across both treatments. All calculations were done using the GenStat statistical package (GenStat, 2009).

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[1] Protosan DS - 1% solution at 1.2 mL of product in 117 mL H_2O/m^2; Virkon S - 3% solution at 2.6 g of product in 94 mL H_2O/m^2; formalin - 18.8 mL of product in 118 mL H_2O/m^2.

Small plot field trialling of entomopathogenic nematodes (*Steinernema carpocapsae*)

Two earth floor broiler houses (house No.s 2 and 5) on a broiler farm at Gatton were used for the small plot study. As for the large scale nematode trial, bedding litter was completely removed from each house at the end of each batch (broiler flock time) and fresh bedding (locally milled wood shavings) was spread onto the floors. The trial was conducted over one broiler batch that commenced at the end of 2009. Disinfectant applications were made to the floors and allowed to dry 1-2 days prior to the applications of treatments. In each of the two houses, just prior to bird placement, two replicates of five different treatments were applied to randomly selected 1.8 m^2 plots of floor in the brooder sections, each plot under a section of feed supply line below three consecutive feed pans (Figure 1). Each 1.8 m^2 plot was demarcated using a frame made from timber doweling (1.8m x 1m) that was placed on the earth floor. The treatment was then applied to the demarcated plot, and then the frame removed. Treatments of nematodes and of fungi were made using 2 L watering cans with 40 cm wide T-shaped nozzles. Treatments of Elector PSP® and Prolong® were made using 1 L atomisers delivering approximately 1 mL per pump spray. Control treatments were undisturbed plots with no water application.

Treatments in the two houses were:

Entomopathogenic fungal treatment (1): *Beauveria bassiana* (strain B27) with 90% viability, applied at 2.75 g of conidia/27.5 mL codacide oil/640mL of H_2O/m^2.

Entomopathogenic nematode treatment (2): *Steinernema carpocapsae* (purchased commercially) applied at 3.91 g (3.88 x 10^6 nematodes)/1000 mL of H_2O/m^2.

Entomopathogenic nematode treatment (3): *Steinernema carpocapsae* (purchased commercially) applied at 3.91 g (3.88 x 10^6 nematodes)/2000 mL of H_2O/m^2.

Elector PSP® treatment (4): 0.22 mL Elector PSP® (i.e. 0.10008 gai spinosad)/33 mL of H_2O/m^2 (modified label rate).

Prolong® treatment (5): 0.2 g Prolong® (i.e. 0.02 gai cyfluthrin)/100 ml of H_2O/m^2 (label rate).

Control treatments (6): no water or control agent applied.

The batch immediately prior to the nematode study batch had received treatments of fungi, Elector PSP®, Prolong® and control to the same plot positions as the current study while the plot positions for the nematode treatments were previously untreated. Samples of litter (62.5 mL per sample) were collected weekly from under the centre feed pan of each treatment plot in the two houses during the time of the field study. These samples were collected by plunging a plastic scoop into the litter until the floor of the house was reached, and then a level scoop of litter was removed. In total, 12 litter samples were collected from each house per week over the study time of one batch (7-8 weeks). Litter samples were transported to the laboratory in vented plastic boxes where live larvae, pupae and adults of *A. diaperinus* were extracted from each sample, counted and discarded.

Effect on virulence of entomopathogenic fungi to lesser mealworm when exposed to three broiler house disinfectants

General methodology

Almost all disinfectant compatibility assays with fungi were undertaken using only *Metarhizium anisopliae* M16 as laboratory stocks of *Beauveria bassiana* were in short supply. Broiler house disinfectants tested were Virkon S®, Protosan DS® and formalin. Field disinfectant surface application

rates were: Protasan DS - 1% solution at 1.2 mL of product in 117 mL H_2O/m^2; Virkon S - 3% solution at 2.6 g of product in 94 mL H_2O/m^2; formalin – 37% solution at 18.8 mL of product in 118 mL H_2O/m^2. Active ingredients in each disinfectant are: Virkon S® - potassium peroxomonosulphate, sulphamic acid and sodium alkyl benzene sulphonate; Protasan DS® - glutaraldehyde; and formalin - formaldehyde, ethanol and formic acid. For all tests, laboratory plastic test boxes (round tapering food containers: 60mm high, bottom radius 40mm, top radius 42 mm: 200 mL) were set up that were roughly analogous to a field situation, i.e. with a simulated broiler house clay floor on the base of each container (i.e. 30 g of pulverised clay mixed with 15 mL of water and spread onto the bottom of each container and allowed to dry overnight at 30°C) with 60 mL of simulated bedding placed on the simulated clay floor. Simulated bedding consisted of 40 mL of clean pine wood shavings and 20 mL of lesser mealworm culture medium (by weight: 76% bran, 17% chicken feed pellets and 7% torula yeast). All treatments of disinfectant and fungi[2] were made to the simulated clay floors prior to the addition of bedding by using an atomizer pump which dispensed *ca* 0.14 mL per single spray. After the addition of bedding into each container, fluon was then applied to the upper inside walls of the containers, and two pieces of sponge (40 x 35 x 4 mm) saturated with water were placed on the surface of the bedding. Finally, 20 mature lesser mealworm larvae were placed onto the bedding in each test box, then sealed in with vented plastic lid and incubated at 30°C and 55% RH. Sponges were re-saturated at 3 and 5 d, and at 7 d test boxes were assessed for larval survival by sieving larvae from bedding. Unless otherwise indicated with the results, the data were analysed by one-way analysis of variance. Pair-wise comparisons between means were done using Fisher's protected Least Significant Difference (LSD) test.

Assay 1: Optimise water volumes for fungal applications

Prior to commencing the disinfectant compatibility assays, a test to optimise water volumes for fungal applications was designed to determine whether fungal formulations applied in varying water volumes influence their virulence to lesser mealworm larvae. Thirty five laboratory test boxes were set up consisting of six fungal treatments and an untreated control (no water treatment), all with five replicates per treatment. For *M. anisopliae* M16 and *B. bassiana* B27 there were three treatments set up for each species with varying amounts of water used for applications to test boxes, i.e. 0.7, 1.4 and 2.1 mL. Therefore, for M16[3], 4.795 x 10^{-2}g of conidia were applied per test box in 0.7, 1.4 and 2.1 mL of H_2O/test box (i.e. 9.59 g of conidia in 140, 280 and 420 mL of H_2O/m^2). In addition, for B27[4], 7.0 x 10^{-3}g of conidia were applied per test box in 0.7, 1.4 and 2.1 mL of H_2O/test box (i.e. 1.4 g of conidia in 140, 280 and 420 mL of H_2O/m^2).

Assay 2: Effect of Virkon S® on virulence of *M. anisopliae*

For Assay 2, 30 laboratory test boxes (five replicates per fungal treatment) were set up in total with five boxes left untreated (control treatment). In 20 boxes, prior to addition of simulated bedding, the simulated clay floor in each test box (*ca* 0.005 m^2) was treated with 0.5mL of a Virkon S® solution (four doses: 5, 15, 25 and 50 x 10^{-3} g/0.5 mL H_2O/test box). These laboratory Virkon S® treatments (i.e. 1, 3, 5 and 10%) were based around the Virkon S® field disinfectant application rate of a 3% solution at 2.6 g of product in 94 mL H_2O/m^2. These treatments were followed immediately by an

[2] The most appropriate conidia to oil ratio used in the formulations for both fungal species was determined by gradually mixing conidia into oil until a limit was reached at which the oil was saturated with conidia but still maintained fluidity and consistency. This limit was determined to be around 0.3-0.4 g of spores/1mL of Codacide® oil and ratios roughly equivalent to this were used in all assays.

[3] M16: the three suspensions were made by mixing 1.37 g of conidia in 3 mL of Codacide® oil and diluted in 20, 40 and 60 mL of H_2O and applied in the test boxes.

[4] B27: the three suspensions were made by mixing 0.2 g of conidia in 1 mL of codacide oil and diluted in 20, 40 and 60 mL of H_2O and applied in the test boxes.

application of *ca* 2.8 mL of fungal suspension[5] (0.0483 g of M16 fungal conidia/test box), which was equivalent to a dose of 9.66 g of conidia/560 mL/m^2. The other five test boxes received a treatment of fungi only.

Assay 3: Effect of Virkon S®, Protosan DS® and formalin on virulence of *M. anisopliae* and *B. bassiana* (increased water volumes with delayed fungal applications)

For this laboratory assay, the virulence of both fungal species was tested against a range of disinfectant doses for the three disinfectants (i.e. 1-fold, 1.5-fold, 3-fold and 5-fold the field disinfectant application rate). Because it was of concern that previous smaller water volumes used for the test boxes might affect the efficiency of the applications of disinfectant and fungi, larger volumes of water were used to apply the disinfectants and fungi for the tests in this assay, while retaining the desired g of product/conidia/m^2. Thus 5 mL of each disinfectant and fungal solution was applied to the simulated clay floor with a bulb pipette about 1-2 min apart. As a baseline, the possible toxicity of the disinfectant to lesser mealworm larvae was also first tested. Therefore, for Sub-assay A, test boxes with larvae were treated with four doses of the three disinfectants using three replicates of each with an untreated control. Sub-assay B were treated with the same four doses of the three disinfectants using three replicates of each with an untreated control, except all had an additional treatment of 0.0483 g of *M. anisopliae* M16 fungal conidia/test box (which was equivalent to our standard dose of 9.66 g of conidia. Sub-assay C was as per Sub-assay B except that the additional treatment was 0.01375 g of *B. bassiana* B27 fungal conidia/test box (which was equivalent to a dose of 2.75 g of conidia/m^2).

Assay 4: Effect of Virkon S®, Protosan DS® and formalin on virulence of *M. anisopliae*

In this assay, the test to measure the effect of the three disinfectants (at a range of disinfectant doses) on the virulence of *M. anisopliae* M16 (as for Assay 3, Sub-assay B, applied at 9.66 g of conidia/m^2 was repeated, except the volumes of water used to apply the disinfectants and the fungal conidia were reverted back to the smaller volumes of suspension applied with an atomiser, and the fungal conidia and culture medium were applied immediately after the disinfectants.

Assay 5: Effect of Virkon S®, Protosan DS® and formalin on virulence of *M. anisopliae* using delayed applications of *M. anisopliae*

This assay was the same as Assay 4, except the disinfectants were only applied at field disinfectant application rates and applications of the fungal conidia suspensions were applied at a range of times (0, 1, 3 and 6 h) following the applications of the disinfectants.

Assay 6: Effect of formalin on virulence of *M. anisopliae* using delayed applications of *M. anisopliae*

This assay was the same as Assay 5, except only formalin was at the field disinfectant application rate was tested and applications of the fungal conidia suspensions were applied at two times (24 and 48 h) following the applications of the disinfectant.

[5] fungal suspension was 3.45 g of M16 conidia in 10 mL of Codacide® oil diluted in 200 mL of H_2O; 2.8 mL of this suspension contained 0.0483 g of M16 conidia.

Small plot field trialling of entomopathogenic fungi (*M. anisopliae* and *B. bassiana*)

As for the small plot nematode trials (section 3.3), the same two earth floor broiler houses (house No.s 2 and 5) on the Gatton broiler farm were used for the fungal small plot studies. The same methodology was also used except that the fungal trials were conducted over seven batches with two or four replicates per treatment, and incorporated treatments of the two fungal species, Elector PSP®, Prolong® and diatomaceaous earth. As per the nematode trials, all treatments were made to the earth floors prior to the start of batches except for some fungal treatments in plot trials 4 and 5 which were applied to litter prior to the start and through the batch. Where possible, the same sample plot positions were maintained for similar treatments in each broiler house in subsequent batches. Diatomaceous earth treatments were applied using a bucket and then raked over the plots.

For each trial, treatment effects on the numbers of lesser mealworms across time were tested by repeated measures analysis of variance, with counts transformed using the log(n+1) transformation before analysis to stabilise the variance. In two cases (trials 1 and 7) the majority of the counts were zero at day 5, so variability was very small and the counts on day 5 were not included in the analyses. In trials 4 and 5 there were extra observations for the control during an initial period, with one of the treatments not applied until after the first 3 or 2 observation times respectively, so repeated measures analyses were done separately for the initial and later periods. In all cases with a significant treatment by time interaction, significance of treatment effects at each time were also tested by analysis of variance. Trials 1 to 5 were each conducted within a single broiler house so the sampling position was the experimental unit for the analyses; trials 6 and 7 were each in two broiler houses, so broiler house was used as a replicate factor with broiler house by treatment combination the experimental unit. Within the analyses for trials 1 to 6 partitioning of the treatment effects and interactions with time was used to test the statistical significance of particular contrasts determined by the treatment structure. In trials 1, 2 and 3 differences between the control and the average of the other treatments and between the other treatments were tested. For trials 4, 5 and 6 the treatment structure included a 2 x 2 factorial within it, with factors Elector and either M16 (trial 4) or B27 (trials 5 and 6), so partitioning was used to test the main effects of these two factors and their interaction. The treatments for trial 7 were unstructured. The GenStat statistical package (GenStat, 2009) was used for all analyses. Effects were tested for statistical significance at the P = 0.05 level.

Specific treatments, broiler houses and batches are described below.

Fungal plot trial 1

This initial trial was conducted in house No. 2 over the batch running from June to August 2008 and included four replicates of each treatment; treatments being 9.6 g of *M. anisopliae* M16 conidia in 32 mL of Codacide® oil in 640 mL of H_2O/m^2, 4.8 g of *M. anisopliae* M16 conidia in 32 mL of Codacide® oil in 640 mL of H_2O/m^2, and untreated without water application.

Fungal plot trial 2

Identical treatments to plot trial 1 except it was conducted in house No. 5 over the September and October 2008 batch.

Fungal plot trial 3

Again, identical treatments to plot trials 1 and 2 in house No. 5, over the November and December 2008 batch except the *M. anisopliae* M16 conidial batch used in this trial had reduced viability (to approximately 60% of its normal viability).

Fungal plot trial 4

Five treatments with four replicates per treatment were set up in house No. 5 over the January, February 2009 batch using *M. anisopliae* M16 with 60% viability and Elector PSP® all applied prior to the commencement of the batch except for Treatment 2.

Treatments were:

Entomopathogenic fungal treatment (1): Conidia applied to the surface of the bedding at 19.2 g of conidia/32 mL Codacide® oil/640 mL of H_2O/m^2.

Entomopathogenic fungal treatment (2): Conidia applied to the surface of the bedding on day 17 of the batch at 19.2 g of conidia/32 mL Codacide® oil/640 mL of H_2O/m^2.

Elector PSP® and entomopathogenic fungal treatments (3): Prior to the start of the batch, 0.22 mL Elector PSP® (i.e. 0.10008 gai spinosad)/33 mL of H_2O/m^2 (modified label rate) applied to the earth floor followed by conidia applied to the surface of the bedding at 19.2 g of conidia/32 mL Codacide® oil/640 mL of H_2O/m^2.

Elector PSP® treatment (4): 0.22 mL Elector PSP® (i.e. 0.10008 gai spinosad)/33 mL of H_2O/m^2 (modified label rate) applied to the earth floor.

Control treatment (5): no water or control agent applied.

Fungal plot trial 5

Five treatments with four replicates per treatment were again set up in house No. 5 except this time using *B. bassiana* (strain B27) (with 90% viability), and Elector PSP® over the March, April 2009 batch, all applied prior to the commencement of the batch except for Treatment 2.

Treatments were:

Entomopathogenic fungal treatment (1): Conidia applied to the surface of the bedding at 2.75 g of conidia/27.5 mL Codacide® oil/640 mL of H_2O/m^2.

Entomopathogenic fungal treatment (2): Conidia applied to the surface of the bedding on day 11 of the batch at 2.75 g of conidia/27.5 mL Codacide® oil/640 mL of H_2O/m^2.

Elector PSP® treatment and entomopathogenic fungal treatment (3): 0.22 mL Elector PSP® (i.e. 0.10008 gai spinosad)/33 mL of H_2O/m^2 (modified label rate) applied to the earth floor followed by conidia applied to the surface of the bedding at 2.75 g of conidia/27.5 mL Codacide® oil/640 mL of H_2O/m^2.

Elector PSP® treatment (4): 0.22 mL Elector PSP® (i.e. 0.10008 gai spinosad)/33 mL of H_2O/m^2 (modified label rate) applied to the earth floor.

Control treatment (5): no water or control agent applied.

Fungal plot trial 6

Six treatments were set up in house No.s 2 and 5 with two replicates of each treatment per house using *B. bassiana* (strain B27) (with 90% viability), Elector PSP®, Prolong® and diatomaceous earth over the July-September 2009 batch. All treatments were made to the earth floors of the houses prior to the commencement of the batch. For Treatments 2 and 3, the conidial suspension was applied first and

allowed to soak into the floor for approximately 10 min after which Elector PSP® or diatomaceous earth was applied.

Treatments were:

Entomopathogenic fungal treatment (1): Conidia applied at 2.75 g of conidia/32 mL Codacide® oil/640 mL of H_2O/m^2.

Elector PSP® treatment and entomopathogenic fungal treatment (2): 0.22 mL Elector PSP® (i.e. 0.10008 gai spinosad)/33 mL of H_2O/m^2 (modified label rate) applied, followed by conidia applied at 2.75 g of conidia/32 mL Codacide® oil/640 mL of H_2O/m^2.

Entomopathogenic fungal treatment and diatomaceous earth treatment (3a, b): Conidia applied at 2.75 g of conidia/32 mL Codacide® oil/640mL of H_2O/m^2 in both houses followed by 0.56 kg diatomaceous earth/m^2 in House 5(a) and 1.39 kg diatomaceous earth/m^2 in House 2 (b).

Elector PSP® treatment (4): 0.22 mL Elector PSP® (i.e. 0.10008 gai spinosad)/33 mL of H_2O/m^2 (modified label rate).

Prolong® treatment (5): 0.2 g Prolong® (i.e. 0.02 gai cyfluthrin)/m^2 (label rate)

Control treatment (6): no water or control agent applied.

Fungal plot trial 7

As per the small plot field trialling of entomopathogenic nematodes using *S. carpocapsae* (Section 3.3).

4. Results

Large scale field trialling of entomopathogenic nematodes (*Steinernema carpocapsae*)

In all houses over the first batch, that received a label rate of Elector® PSP (480 g/L spinosad), i.e. 200 mL applied in 50-80 L of water per house, lesser mealworm numbers varied greatly between houses (Table 1). Remarkably low numbers were sampled from untreated house No. 4 which had a relatively hard floor constructed from a concrete pavement base soil stabiliser (Weslig®). For the second batch of the study (Figure 2), insect numbers increased in three of the four nematode treated houses, and in house No. 2 which was untreated. As for the first batch, again almost no insects were collected from house No. 4 during the second batch. The only two houses that showed a decline in lesser mealworm numbers from the first to the second batch were house No. 3, which had received a modified spinosad application and house No. 5 (a narrow band nematode application). In the other spinosad treated house, No. 1, recorded insect numbers were roughly equivalent for the two batches.

Table 1 Total live *Alphitobius diaperinus* collected from eight broiler houses over two batches; Batch 2 received 4 separate treatments; all houses had earth floors except House 4 which had a concrete stabilised floor

House No.	Batch 2 treatments	Total insects collected	
		Batch 1[+]	Batch 2
1	spinosad (A)	3115	3073
2	control (A)	2500	7173
3	spinosad (B)	11194	4512
4	control (B)	34	5
5	nematode 1 (A)	8101	5792
6	nematode 2 (B)	5215	9696
7	nematode 2 (A)	6980	11361
8	nematode 1 (B)	9146	10791

[+]For Batch 1 all houses received whole floor applications of spinosad using 200 mL of Elector PSP® (480 g/L) applied in 50-60 L of water

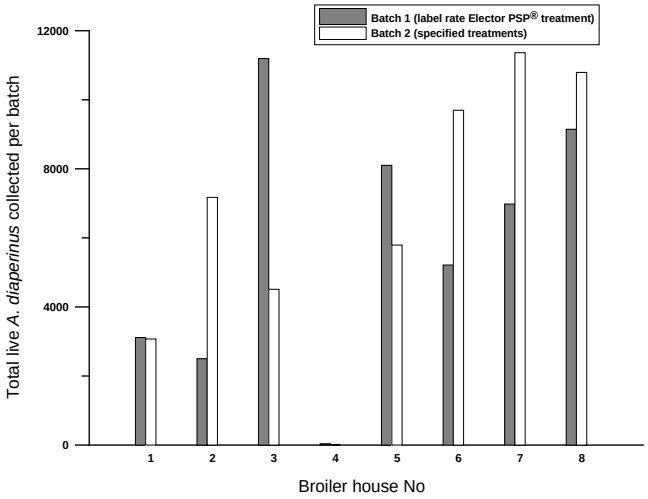

Figure 1 Total live *Alphitobius diaperinus* collected from eight broiler houses over two batches; Batch 2 received 4 separate treatments; all houses had earth floors except House 4 which had a concrete stabilised floor

Effect on virulence of entomopathogenic nematodes (*Steinernema carpocapsae*) to lesser mealworm when exposed to three broiler house disinfectants

Very little mortality was recorded in the two negative control treatments which did not have nematodes added to the simulated bedding, i.e. water and disinfectant only treatments (Table 2). The control that included nematodes gave mortality of larvae ranging from 33.5 to 80%. The results showed no significant differences in mortalities between the Control (with nematodes only), nematodes plus Protosan® and nematodes plus Virkon® treatments, but these had significantly higher mortalities than with no nematodes and with nematodes plus formalin, with the difference between these latter two treatments not significantly different.

Table 2 Mean % mortality of large larvae of *Alphitobius diaperinus* after treatments with entomopathogenic nematodes (*Steinernema carpocapsae*) in combination with three disinfectants. Control treatments included two negative controls

	Entomopathogenic nematode assays		
Assay No.	1	2	3
No. of replicates	3	3	3
Nematode dose (nematodes/2.5 mL/m^2) ex nematode colony	5950	4925	3090
	Mean % mortality of lesser mealworm larvae		
Negative control (H_2O only)	0	0	3.3
Negative control (disinfectant only)	0	0	0
Control (nematodes only)	33.3	60	80
Treated with Protosan DS* and nematodes	76.7	53.3	60
Treated with Virkon S* and nematodes	66.7	53.3	53.3
Treated with formalin* and nematodes	6.7	13.3	6.7

*Disinfectant doses were based on field disinfectant surface application rates; Field disinfectant surface application rates were: Protosan DS - 1% solution at 1.2 mL of product in 117 mL H2O/m2; Virkon S - 3% solution at 2.6 g of product in 94 mL H2O/m2; formalin - 18.8 mL of product in 118 mL H2O/m2

Table 3 Mortality of larvae of *Alphitobius diaperinus* recorded after exposure to *Steinernema carpocapsae* in laboratory test boxes with and without three disinfectants

Treatment	**Unranked mean**	**Back-transformed mean (% scale)**
No nematodes	-5.192_a	0.55
Control	0.314_b	57.8
Protosan DS	0.548_b	63.36
Virkon S	0.314_b	57.8
Formalin	-1.695_a	15.51

NB. Control was nematodes without disinfectant. Means with the same subscript are not significantly different at the P = 0.05 level; LSD = 2.774

Small plot field trialling of entomopathogenic nematodes (*Steinernema carpocapsae*)

See: Section 4.5.7. Fungal plot trial 7.

Effect on virulence of entomopathogenic fungi to lesser mealworm when exposed to three broiler house disinfectants

Optimise water volumes for fungal applications (Assay 1)

The results of the tests indicated that the mean % larval mortality induced by exposure to fungal did not seem related to the volumes of water used to apply the conidia, as both fungal species showed no significant difference in mortality between volumes of water (Table 4) although there was a significant treatment effect for both fungal species when compared to the control. What the results also showed was that *M. anisopliae* M16 treatments produced significantly higher mortality in almost all cases than those recorded for *B. bassiana* B27 ($P<0.001$).

Table 4 Mean % mortality of larvae of *Alphitobius diaperinus* recorded after exposure to *M. anisopliae* M16 and *B. bassiana* B27 applied in a range of water volumes to a simulated earth floor in laboratory test boxes

Fungal treatment	Water volume applied (mL)	Mean % larval mortality*
0	0	0_{d}
M16	0.7	17.2_{a}
	1.4	16.6_{a}
	2.1	13.8_{ab}
B27	0.7	8.6_{c}
	1.4	3.8_{d}
	2.1	11.2_{bc}

NB. Means with the same subscript are not significantly different at the P = 0.05 level; LSD = 3.93

Effect of Virkon S® on virulence of *M. anisopliae* (Assay 2)

The results of this test showed that Virkon S® had no significant effect on the virulence of *M. anisopliae* M16 to lesser mealworm (Table 5). The test also indicated that the fungal application, with and without disinfectant showed a significant treatment effect, with all fungal treatments giving significantly higher mean % lesser mealworm mortalities than the untreated control ($P<0.001$).

Table 5 Mean % mortality of larvae of *Alphitobius diaperinus* recorded after exposure to *M. anisopliae* M16 applied with a range of Virkon S® concentrations to a simulated earth floor in laboratory test boxes

Fungal treatment	Disinfectant	Mean % larval mortality*
0	0	4.2 $_{b}$
M16	0	19.2 $_{a}$
M16	Virkon 1%	19.8 $_{a}$
M16	Virkon 3%	18.8 $_{a}$
M16	Virkon 5%	19.6 $_{a}$
M16	Virkon 10%	20.0 $_{a}$

NB. Means with the same subscript are not significantly different at the P = 0.05 level; LSD = 1.563

Effect of Virkon S®, Protosan DS® and formalin on virulence of *M. anisopliae* and *B. bassiana* (increased water volumes with delayed fungal applications) (Assay 3)

Exposing lesser mealworm larvae to the three disinfectants (Sub-assay A) produced no significant mortality, i.e. not significantly different to the untreated (P = 0.387). For Sub-assay B (Table 6) which tested the effect of the three disinfectants to the virulence of *M. anisopliae* M16 there was a significant treatment effect using formalin (P<0.001). Using all concentrations of formalin reduced the efficacy of M16 to a mean % mortality not significantly different to the untreated level. The other two disinfectants, in particular Virkon S® had no significant effect on the virulence of the fungi. In contrast, in Sub-assay C the virulence of *B. bassiana* was significantly affected by all the disinfectants at all doses (Table 7), in particular by formalin. Overall there was a significant treatment effect (P<0.001) with the treatment using only fungi significantly different to all other treatments, and the untreated and formalin treatments significantly different to all other treatments.

Table 6 Mean % mortality of larvae of *Alphitobius diaperinus* recorded after exposure to *M. anispliae* M16 applied with a range of Virkon S®, Protosan DS® and formalin concentrations to a simulated earth floor in laboratory test boxes (Sub-assay B)

Fungal treatment	Disinfectant	Mean % larval mortality*
0	0	2.00 $_{e}$
M16	0	20.00 $_{a}$
M16	Virkon x1	18.00 $_{abc}$
M16	Virkon x1.5	15.67 $_{bcd}$
M16	Virkon x3	18.00 $_{abc}$
M16	Virkon x5	17.67 $_{abc}$
M16	Protosan x1	13.33 $_{d}$
M16	Protosan x1.5	18.67 $_{ab}$
M16	Protosan x3	14.67 $_{cd}$
M16	Protosan x5	15.67 $_{bcd}$
M16	Formalin x1	1.67 $_{e}$
M16	Formalin x1.5	1.33 $_{e}$
M16	Formalin x3	1.00 $_{e}$
M16	Formalin x5	1.33 $_{e}$

NB. Means with the same subscript are not significantly different at the P = 0.05 level; LSD = 3.345

Table 7 Mean % mortality of larvae of *Alphitobius diaperinus* recorded after exposure to *B. bassiana* B27 applied with a range of Virkon S®, Protosan DS® and formalin concentrations to a simulated earth floor in laboratory test boxes (Sub-assay C)

Fungal treatment	Disinfectant	Mean % larval mortality*
0	0	3.33 e
B27	0	16.67 a
B27	Virkon x1	7.33 cd
B27	Virkon x1.5	11.67 b
B27	Virkon x3	9.00 bcd
B27	Virkon x5	9.00 bcd
B27	Protosan x1	9.33 bcd
B27	Protosan x1.5	6.67 d
B27	Protosan x3	10.00 bc
B27	Protosan x5	10.00 bc
B27	Formalin x1	1.33 ef
B27	Formalin x1.5	0 f
B27	Formalin x3	1.67 ef
B27	Formalin x5	0.33 ef

NB. Means with the same subscript are not significantly different at the P = 0.05 level; LSD = 3.223

Effect of Virkon S®, Protosan DS® and formalin on virulence of *M. anisopliae* (Assay 4)

This assay (using *M. anisopliae* M16), which reverted back to smaller dose volumes for the disinfectants and fungi and no delay for the fungal application gave similar results to the previous assay (Table 8). There was a significant treatment effect (P<0.001) for formalin doses whose mean % mortalities were not significantly different to the untreated but were significantly different to all other treatments. The mortalities produced by applications of M16 fungi only, were not significantly different to the fungi applied with all doses of Virkon S® and Protosan DS®.

Table 8 Mean % mortality of larvae of *Alphitobius diaperinus* recorded after exposure to *M. anispliae* M16 applied with a range of Virkon S®, Protosan DS® and formalin concentrations to a simulated earth floor in laboratory test boxes

Fungal treatment	Disinfectant	Mean % larval mortality*
0	0	4.67 $_{c}$
M16	0	17.67 $_{a}$
M16	Virkon x1	18.67 $_{a}$
M16	Virkon x1.5	16.67 $_{a}$
M16	Virkon x3	18.33 $_{a}$
M16	Virkon x5	15.33 $_{a}$
M16	Protosan x1	18.67 $_{a}$
M16	Protosan x1.5	18.67 $_{a}$
M16	Protosan x3	16.33 $_{a}$
M16	Protosan x5	15.33 $_{a}$
M16	Formalin x1	4.67 $_{c}$
M16	Formalin x1.5	9.33 $_{fb}$
M16	Formalin x3	8.33 $_{bc}$
M16	Formalin x5	10.67 $_{b}$

NB. Means with the same subscript are not significantly different at the P = 0.05 level; LSD = 4.137

Effect of Virkon S®, Protosan DS® and formalin on virulence of *M. anisopliae* using delayed applications of *M. anisopliae* (Assay 5)

This assay which delayed the application of fungal suspension (using *M. anisopliae* M16) also gave a significant treatment effect for formalin (P<0.001) with the mean % mortality produced by the addition of formalin significantly different to all other treatments including the untreated. The mean % mortalities from applications of M16 fungi only were not significantly different to the fungi applied with Virkon S® and Protasan DS®. When analysed as a two way ANOVA with delay as a treatment factor, the analysis indicated that the interaction between the applied treatment and the delay was not significant (P = 0.80). The delay was also not significant (P = 0.40).

Effect of formalin on virulence of *M. anisopliae* using delayed applications of *M. anisopliae* (Assay 6)

For this assay, the delay after formalin for the fungal application was extended out to 24 and 48 h. As for the other assays with formalin there was a significant treatment effect (P<0.001). However, when analysed as a two way ANOVA with delay as a treatment factor, the analysis indicated that the interaction between the applied treatment and the delay was not significant (P = 0.08). The delay though (24 and 48 h) was significant (P = 0.002) (Table 9).

Table 9 Mean % mortality of larvae of *Alphitobius diaperinus*: formalin was first applied to a simulated earth floor in laboratory test boxes following by delayed applications (24 & 48h) of *M. anispliae* M16

Treatment	Mean % larval mortality*
nil	0.83 $_{b}$
M16 only	15.33 $_{a}$
M16 and formalin	15.33 $_{a}$

NB. Means with the same subscript are not significantly different at the P = 0.05 level; LSD = 2.533

Delay (hr)	Mean % larval mortality*
24	12.33 $_{a}$
48	8.67 $_{b}$

*Larval mortalities are presented as transformed means (log $^{x+1}$); NB. Means with the same subscript are not significantly different at the P = 0.05 level; LSD = 4.137

Small plot field trialling of entomopathogenic fungi (*M. anisopliae* and *B. bassiana*)

Fungal plot trial 1

For this plot trial (Table 10, Figure 2), the ANOVA test of differences between means over time for the control and the two *M. anisopliae* treatments indicated a significant difference (P<0.05) between the mean of the two fungal treatments and the mean for untreated, but no significant difference between the two fungal treatments. The corresponding ANOVA analysis of interactions with time indicated that the differences between the untreated and the average for the fungal treatments varied with time, with the LSD = 2.32 (transformed scale) for testing between means at any time. Using this LSD, there was only a significant difference between the control and either fungal treatment at 20d.

Table 10 Table of means (for all times) for *Alphitobius diaperinus* collected over a single flock time within one broiler house at separate treated plot areas (Plot trial 1).

Treatments were: *Metarhizium anisopliae* (M16) applied at 4.8 and 9.6g/m^2 and an untreated control; LSD (5%) = 2.32 (transformed scale)

Time (d)		**5**	**13**	**20**	**27**	**34**	**41**	**48**	**56**	**mean**
Treatment	Control	0.85	2.05	6.02	5.65	6.42	4.22	3.13	5.49	4.23
	M16 (4.8g/m^2)	0	1.04	2.27	4.64	5.0	3.55	5.03	4.36	3.24
	M16 (9.6g/m^2)	0	0	1.87	3.84	5.41	4.27	4.64	3.73	2.97
	Mean	0.28	1.03	3.39	4.71	5.61	4.01	4.26	4.53	3.48

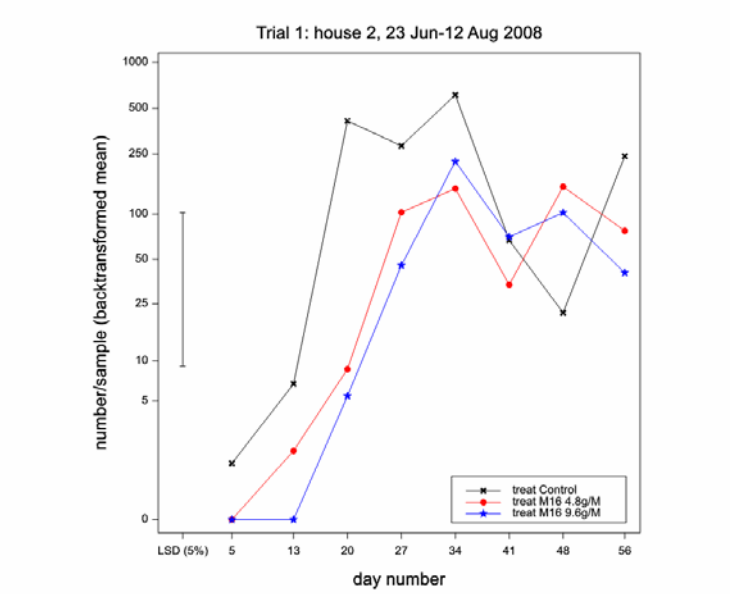

Figure 2 **Means (back transformed) for *Alphitobius diaperinus* collected over a single flock time (56 d) within one broiler house (house No. 2) at treated plot areas (Plot trial 1).**

Treatments were: *Metarhizium anisopliae* (M16) applied at 4.8 and 9.6 g/m^2 and an untreated control; vertical bar is the LSD (5%) = 2.32 (transformed scale) for testing between treatments at each time.

Fungal plot trial 2

This plot trial (Figure 3), which was identical to plot trial 1 except that it was conducted in house No. 5, showed no significant difference between treatment means (P = 0.958) and treatments over times (P = 0.279), i.e. the effect of *Metarhizium anisopliae* (M16) applied at 4.8 and 9.6 g/m^2 was not significantly different to the untreated control.

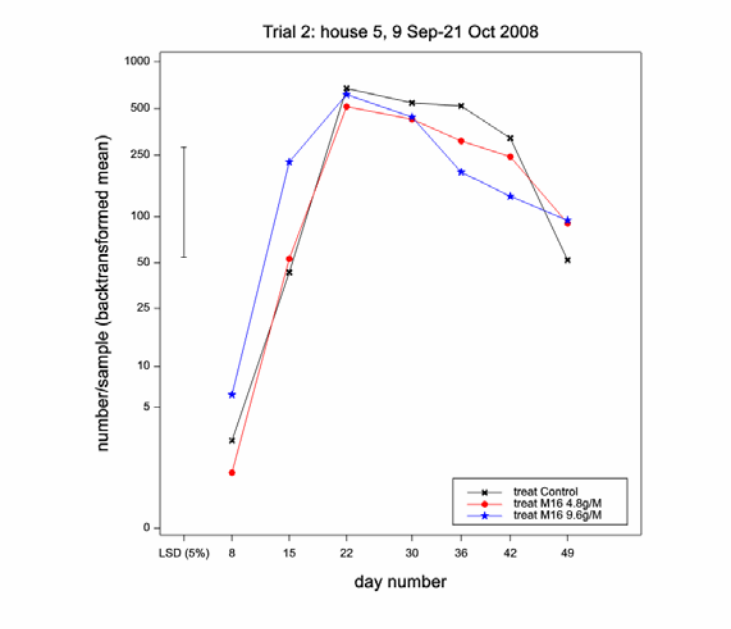

Figure 3 **Means (back transformed) for *Alphitobius diaperinus* collected over a single flock time (56 d) within one broiler house (house No. 5) at treated plot areas (Plot trial 2).**

Treatments were: *Metarhizium anisopliae* (M16) applied at 4.8 and 9.6 g/m^2 and an untreated control; vertical bar is the LSD (5%) = 1.63 (transformed scale) for testing between treatments at each time.

Fungal plot trial 3

Plot trial 3 (in house No. 5) (Figure 4) was a repeat of plot trials 1 and 2 except the *M. anisopliae* M16 conidial batch used had reduced viability. This trial showed no significant difference between treatment means (P = 0.318) and treatments over times (P = 0.136), i.e. the effect of *Metarhizium anisopliae* (M16) applied at 4.8 and 9.6 g/m^2 was not significantly different to the untreated control.

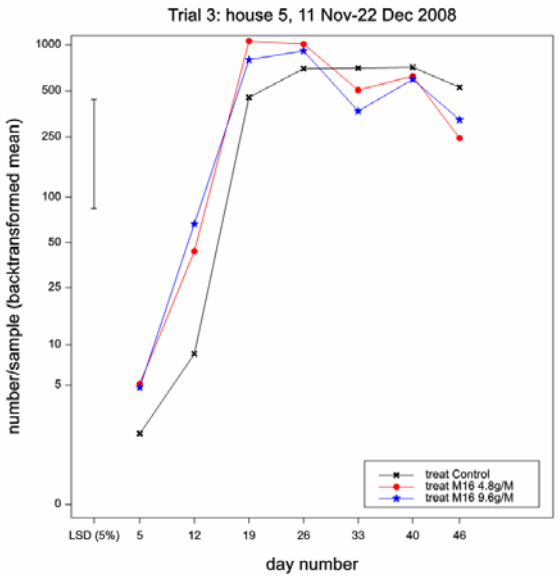

Figure 4 Means (back transformed) for *Alphitobius diaperinus* collected over a single flock time (56 d) within one broiler house (house No. 5) at treated plot areas (Plot trial 3).

Treatments were: *Metarhizium anisopliae* (M16) applied at 4.8 and 9.6 g/m^2 and an untreated control; vertical bar is the LSD (5%) = 1.64 (transformed scale) for testing between treatments at each time.

Fungal plot trial 4

For plot trial 4, five treatments with four replicates per treatment were set up in house No. 5 using *M. anisopliae* M16 (with 60% viability) and Elector PSP®, all applied prior to the commencement of the batch except for a fungal treatment applied at day 17. The analysis indicated no significant treatment effect after day 17 but prior to this there was a significant treatment M16 effect at day 16 (Figure 5, Table 11). At day 16, the means of M16 and the combination of M16+Elector PSP® were significantly different to the means of only Elector PSP® and the control, of which these latter two were not significantly different to each other.

Table 11 Table of back transformed means (for all times) for *Alphitobius diaperinus* collected over a single flock time within one broiler house at separate treated plot areas (Plot trial 4).

Treatments used *Metarhizium anisopliae* (M16) applied at 19.2 g/m^2 (at d-0 and d-17), Elector PSP and untreated; approximate LSD (5%) = 1.91 (transformed scale)

Time (d)	2	9	16	23	30	37	44	Mean
	Treatment							
Control	1.5	1.8	859.8	1041.0	584.1	197.9	191.0	61.0
M16	0.2	17.1	213.4	553.4	429.3	151.7	223.9	86.0
Elector PSP	0.3	3.8	939.9	798.7	589.1	210.4	358.1	110.4
M16 + Elector PSP	1.6	0	152.0	803.5	598.7	173.2	303.7	71.1
M16 at day 17	-	-	-	675.0	486.0	192.7	214.6	341.4
Mean	0.9	2.7	468.7	757.6	532.9	184.0	251.1	92.5

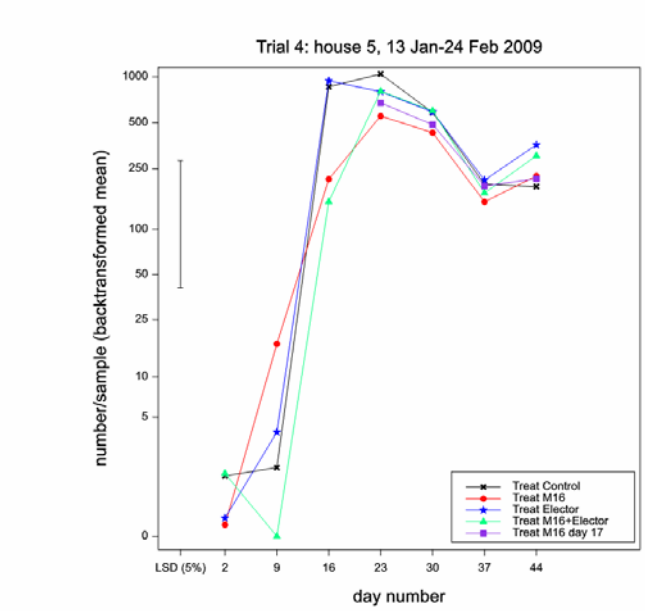

Figure 5 Means (back transformed) for *Alphitobius diaperinus* collected over a single flock time (56 d) within one broiler house (house No. 5) at treated plot areas (Plot trial 4).

Treatments were: *Metarhizium anisopliae* (M16) at reduced viability applied at 19.2 g/m^2, with and without Elector PSP® and an untreated control; vertical bar is the approximate LSD (5%) = 1.91 (transformed scale) for testing between treatments at each time.

Fungal plot trial 5

Plot trial 5 was the same as plot trial 4 except *Beauvaria bassiana* (strain B27) (with 90% viability) was used instead of *M. anisopliae*. The results were very similar to plot trial 4 except in that from day 17 onwards there were no treatment effects for B27 or Elector PSP®. In contrast to plot trial 4 there was a very strong effect of Elector PSP® at day 11. The fungal application showed no significant effect at any time (Figure 6, Table 12).

Table 12 Table of back transformed means (for all times) for *Alphitobius diaperinus* collected over a single flock time within one broiler house at separate treated plot areas (Plot trial 5).

Treatments used *Beauvaria bassiana* (B27) applied at 2.75 g/m^2 (at d-0 and d-11), Elector PSP and untreated; approximate LSD (5%) = 1.46 (transformed scale)

Time (d)	5	11	19	26	33	40	47	Mean
				Treatment				
Control	15.1	135.9	908.9	976.4	899.6	108.0	60.4	142.7
B27	2.1	52.6	670.6	907.8	608.5	142.1	37.2	118.2
Elector PSP	2.9	7.6	604.3	747.0	820.9	167.7	134.3	115.1
B27 + Elector PSP	2.6	6.2	434.8	612.7	514.7	64.6	195.5	88.6
B27 at day 11	-	-	616.4	887.7	834.3	76.3	130.5	340.7
Mean	5.5	35.2	629.3	815.3	719.6	105.0	95.1	135.6

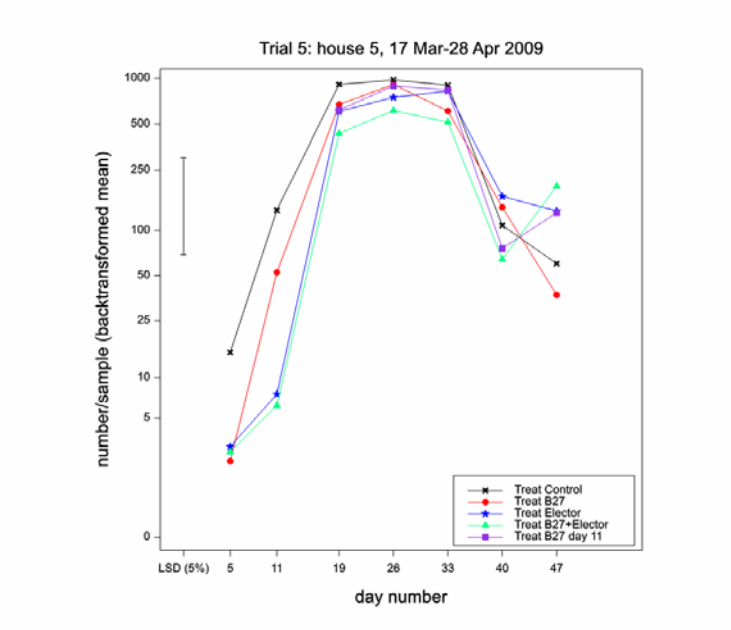

Figure 6 Means (back transformed) for Alphitobius diaperinus collected over a single flock time (56 d) within one broiler house (house No. 5) at treated plot areas (Plot trial 5).

Treatments were: Beauvaria bassiana (B27) (with 90% viability) applied at 2.75 g/m2 with and without Elector PSP® and an untreated control; vertical bar is the approximate LSD (5%) = 1.46 (transformed scale) for testing between treatments at each time.

Fungal plot trial 6

Plot trial 6 included a broader range of treatments. *Beauvaria bassiana* (as for plot trial 5) was applied alone and with Elector PSP® and diatomaceous earth. In addition, stand alone treatments of Elector PSP® and Prolong® (cyfluthrin) were also applied. For this trial there was no significant difference between treatments including the control (Figure 7).

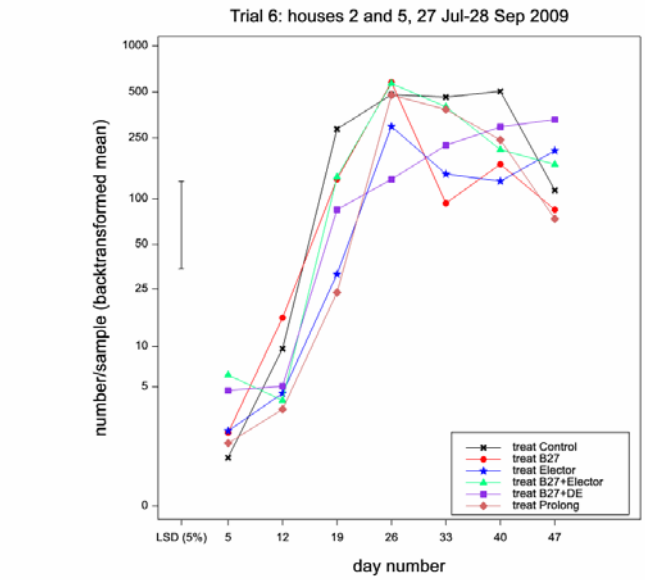

Figure 7 Means (back transformed) for *Alphitobius diaperinus* collected over a single flock time (56 d) within one broiler house (house No. 5) at treated plot areas (Plot trial 6).

Treatments were: *Beauvaria bassiana* (B27) (with 90% viability) applied at 2.75 g/m^2 with and without Elector PSP®, with diatomaceous earth, and Prolong® and an untreated control; vertical bar is the LSD (5%) = 1.31 (transformed scale) for testing between treatments at each time.

Fungal plot trial 7

For this plot trial, *B. bassiana*, *S. carpocapsae*, Elector PSP® and Prolong® were applied as well as a control treatment. As for the treatments in plot trial 6, there was no significant difference between treatments including the control (Figure 8).

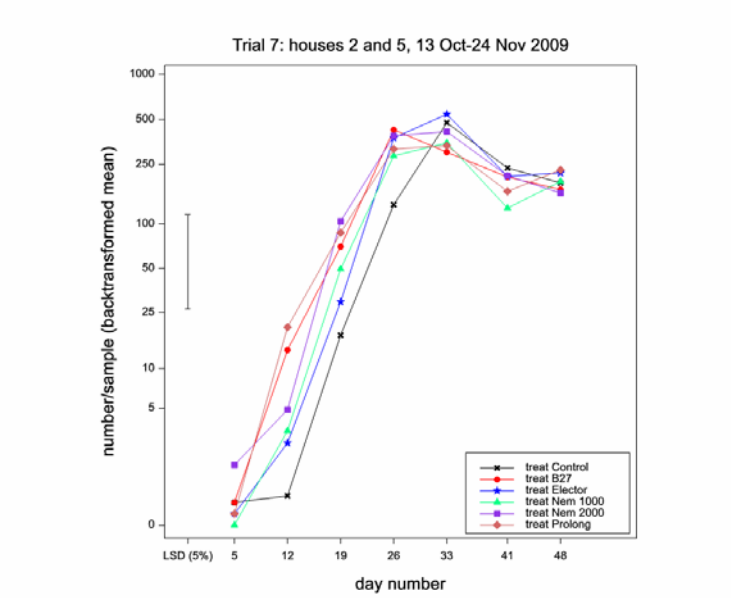

Figure 8 Means (back transformed) for *Alphitobius diaperinus* collected over a single flock time (56 d) within one broiler house (house No. 5) at treated plot areas (Plot trial 7).

Treatments were: *Beauvaria bassiana* (B27) (with 90% viability) applied at 2.75 g/m^2, 3.91g/m^2 *Steinernema carpocapsae*, Elector PSP®, Prolong® and an untreated control; vertical bar is the LSD (5%) = 1.44 (transformed scale) for testing between treatments at each time.

5. Implications

The results from the laboratory trials which assessed the effect of disinfectants on the virulence of entomopathogenic nematodes and fungi (M16 and B27) indicated that almost all treatments of Protosan DS® and Virkon S® tested (including field application rates) had no significant effect on the three agents' virulence even when applied concurrently. Formalin consistently had a significant effect on the virulence of the three agents but this effect was much less 24 hr and 48 hr later, when the toxicity of the formalin had waned. The large scale applications of entomopathogenic nematodes to broiler houses trialled in this study overall gave disappointing results. This was in contrast to the very positive data that came from previous laboratory studies. Apart from house No. 5 which received a narrow band nematode application under the feed supply lines and the two spinosad treated houses (house No.s 1 & 3) almost all the remaining houses saw an increase in total beetle numbers from the first to the second batch (i.e. a control untreated house and the other three nematode treated houses). Considering the high variability in beetle numbers that normally occurs between broiler houses and that the treatment was only run over one batch, nothing noteworthy can be derived from the lower beetle numbers recorded in the nematode treated house No. 5. What was notable was the extremely low numbers collected in house No. 4 which had a relatively hard floor constructed from a concrete pavement base soil stabiliser known as Weslig® which was installed in 2007.

Trials using small plots under feed pans treated with fungi, in general gave results that were inconclusive. These trials were designed to offer a quick and cost effective look at the relative efficacy of fungi and nematodes. During the development of the protocol for the small plot trials, reservations were held that measurements of agents' efficacy would not be appropriate due to the high degree of movement of lesser mealworms in broiler houses that typically occurs. In essence, the measurement of the effectiveness of discrete applications of control agents applied under feed pans can be compromised by the active movement of lesser mealworm larvae, mostly travelling to under feed pan areas. The data from the small plot fungal trials were mostly inconsistent and therefore unpredictable and did not mirror the results of comparable laboratory tests. Therefore, despite results of laboratory fungal trials often not providing complete agreement with results of analogous field fungal studies, in this case, it was felt that the levels of inconsistency and disaccord recorded in the small plot fungal trials were predominately related to insect movement. Hence for any further earnest field trialling of fungal agents it would best be done on a whole-house basis or isolating treated plots with deeply imbedded barriers.

6. Recommendations

Management of lesser mealworm (Darkling Beetle) remains a difficult task, not only in using the appropriate application of insecticides but in the management of insecticide resistance. This trialling of biological agents has provided no conclusive results to base any future action on. Therefore, to go forward with the most realistic and science based approach to management of lesser mealworm in Australian broiler houses it is firstly recommended not to use any further resources for the testing of diatomaceous earth, and entomopathogenic fungi and nematodes as beetle control agents. In addition, due to the adverse effect that continued applications of cyfluthrin is having on the cross resistance status of other pyrethroids currently being tested, it is also recommended to quickly reduce cyfluthrin use in Australian broiler houses and adopt spinosad as an interim treatment until a new combination of effective chemicals, which are currently being researched, are available to the producer.

References

GenStat (2009). GenStat for Windows, 12th edition. VSN International Ltd.

Trialling Biological Agents for the Management of Lesser Mealworm in Australian Broiler Houses

by Trevor A Lambkin

Publication No. 11/033

Lesser mealworm or darkling beetle, *Alphitobius diaperinus* are common insect pests of broiler houses throughout the world.

As they can function as vectors for a large number of avian diseases and parasites and can carry food borne diseases, large lesser mealworm populations pose significant threats to broiler flock health and the production of safe food. They are also structural pests of broiler houses, causing damage to compacted earth floors, and ceiling and wall insulation. This report describes the trialling of biological agents for the management of lesser mealworm in broiler houses.

Cover photo: Lesser mealworm or darkling beetle, *Alphitobius diaperinus* are common insect pests of broiler houses throughout the world

Contact RIRDC:
Level 2
15 National Circuit
Barton ACT 2600

PO Box 4776
Kingston ACT 2604

Ph: 02 6271 4100
Fax: 02 6271 4199
Email: rirdc@rirdc.gov.au
web: www.rirdc.gov.au
Bookshop: 1300 634 313

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