Administration of *Brevibacillus laterosporus* spores as a poultry feed additive to inhibit house fly development in feces: A new eco-sustainable concept

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ABSTRACT The success of a microbial pesticide application against house flies developing in manure should accomplish the uniform mixing of active ingredients with this breeding medium, thus enhancing residual effects. The oral administration of the entomopathogenic bacterium *Brevibacillus laterosporus* to caged poultry species allows the homogeneous incorporation of its active ingredients with fly breeding media. Feces from treated broilers or hens show toxicity against exposed fly adults and larvae. Insecticidal effects are concentration-dependent with a lethal median concentration (LC_{50}) value of 1.34×10^8 and 0.61×10^8 spores/g of feces for adults and larvae, respectively. Manure toxicity against flies was maintained as long as chickens were fed a diet containing adequate concentrations of *B. laterosporus* spores. Toxicity significantly decreased after spore administration to birds was interrupted. When poultry diet contained 10^{10} spores/g, mortality of flies reared on feces exceeded 80%. The use of *B. lateroporus* spores as a feed additive in poultry production systems fostering a more integrated approach to farming is discussed.

Key words: house fly, *Musca domestica*, *Brevibacillus laterosporus*, poultry, feed additive

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INTRODUCTION

Musca domestica L. (Diptera: Muscidae), also known as the house fly, is a disease carrier and an annoyer of primary importance in livestock environments, including poultry houses, where immature breeding is facilitated by the production of abundant animal feces (Axtell, 1985).

Due to the problems connected with high density of fly populations, their management is normally required, especially during favorable conditions for flies (Moon, 2002). Over the years, the containment of fly infestations has relied on a wide variety of chemical insecticides with variable modes of action. However, due to the rapid development of fly resistance to chemical molecules (Scott et al., 2000; Rinkevich et al., 2012) and to the risks for the environment connected with the use of conventional insecticides, the implementation of effective integrated pest management strategies is strongly needed. This presumes the availability of alternative low-impact active ingredients to be used in integration or rotation with chemicals.

Different are the efforts made for studying and developing more environmentally responsible fly management tools, such as different kinds of traps and adult baits (Geden et al., 2009), the release of pupal parasitoids and various bioinsecticides, including botanicals and microbials (Geden, 2012). Among the latter, there are diverse microbial control agents like the nematodes belonging to Steinernematid and Heterorhabditid families (Renn, 1998), fungi as *Entomophthora muscae*, *Beauveria bassiana*, and *Metarhizium anisopliae* (Geden et al., 1993; Darwish and Zayed, 2002; Mishra et al., 2011), bacteria as *Bacillus thuringiensis* (Johnson et al., 1998), and viruses such as MdSGHV, the agent of salivary gland hypertrophy (Lietze et al., 2011).

More recently, the potential of the bacterium *Brevibacillus lateropsorus* as an emerging entomopathogen against the house fly has been highlighted (Ruiu et al., 2013). Spore suspensions of this microorganism cause mortality or sublethal effects on house fly larvae and adults (Ruiu et al., 2006, 2007b). Suppression of immature fly development in manure breeding sites was obtained after the application of *B. laterosporus*-based experimental formulations in comparative experiments with plant-derived insecticides (Ruiu et al., 2008, 2011).

The optimization of formulation features and delivery methods is a major challenge for all microbial pesticides (Satinder et al., 2006). In the case of house fly larvae, a suitable formulation application should accomplish the uniform mixing of spores and manure, thus enhancing insecticidal effects. In this context, the oral administration of entomopathogenic bacteria, such as *Bacillus*

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thuringiensis, to livestock animals to cumulate in feces has previously been proposed (Mwamburi et al., 2011).

The purpose of the present work was to investigate the potential of *B. laterosporus* spores as a feed additive for poultry species, to study an alternative way of application that may ensure a constant and more homogeneous incorporation of its active ingredients with fly breeding media.

MATERIALS AND METHODS

Bacterial Preparations

Brevibacillus laterosporus strain UNISS 18 deposited at the NCIMB Ltd. culture collection (Aberdeen, UK) was routinely grown in conical flasks containing Luria-Bertani (**LB**) broth at 30°C with shaking at 180 rpm. Pure spore suspensions were obtained through culture synchronization procedures involving heat-activation (70°C for 30 min) of the first spore inoculum followed by a preculture in LB broth as described in Ruiu et al. (2007b).

Spores were harvested by 3 cycles of centrifugation-washing in sterile water spinning at 10,000 × *g* for 10 min at 4°C. Suspension purity and quantification was determined under a phase microscope using a Thoma chamber (E. Hartnack, Berlin, Germany). Stock suspensions with a standard concentration of 10^{10} spores/mL were stored at −20°C until use.

Chickens and Housing

Experiment series were conducted with Leghorn broilers and hens, having an age of 3 and 51 wk, respectively. Three days before each experiment started, a new batch of 20 to 30 chickens was provided by Foddi Luigi Azienda Agricola (Gonnosfanadiga, Italy). Birds were delivered to the experiment station, randomly kept in poultry cages stacked in rows in groups of 4, and maintained inside a paddock covered by roofing. Each cage, measuring 28.5 × 25.5 × 42.0 cm, hosted a single bird that was given ad libitum access to water and food provided employing individual drinking bowls and feeder trays, respectively. Chicken feed (Battovo Vegetale Farina, Martini S.p.A., Longiano, FC, Italy) contained all the nutrients to support adequate growth according to the age, including vitamins and minerals.

Each feeding experiment started after birds adapted for 3 d to these conditions.

The experimental animal protocol has been approved by the Animal Care Committee (CIBASA) of the University of Sassari (Italy).

Poultry Feeding and Feces Collection

Several studies were conducted to collect feces from differently treated and untreated birds. Depending on the experiment, *B. laterosporus* spores were administered to new batches of birds via either oral gavage or as an additive mixed into solid feed. Feces were then analyzed and employed in bioassays with flies. The following 4 experiments were conducted.

Experiment 1. The objective of this experiment was to produce fresh feces to inoculate in vitro with bacterial spores. A group of untreated broilers (4 birds) was maintained for 2 wk as previously described with the sole objective to collect excreta in an aluminum tray placed underneath each cage. Daily collected manure was brought to the laboratory for the preparation of the first bioassays with flies, in which manure was directly mixed with different amounts of *B. laterosporus* spores.

Experiment 2. The objective of this experiment was to test the ability of bacterial spores to survive the digestive tract and determine if live bacteria remained toxic to flies after excretion. Spore suspensions (1 mL) at a concentration of 10^9 spores/mL were orally administered to treated broilers (oral gavage method) every day for 10 consecutive days using a pipette, while saline was administered to control birds. At the same time, water and solid food (nontreated) continued to be provided ad libitum to all birds. For 2 wk from the beginning of the experiment, a new aluminum tray was placed underneath each cage every day to collect daily excreta to be employed in fly bioassays. In this way, feces were collected both during the first 10 d (treatment period) and after birds did not get any treatment (post-treatment period) to test the residual spore effects.

Experiment 3. The objective of this experiment was to determine the effects of different bacterial concentrations in broilers diet on the toxicity of their excreta against flies. A predetermined amount of *B. laterosporus* spore suspension was homogeneously mixed into chicken solid feed (feed additive method) with a kneader to obtain the following range of concentrations that were administered to broilers: 10^8, 10^9, and 10^{10} spores/g of diet. Birds were observed to consume the administered feed that was replaced every day with newly treated feed. No bacterial spores were included in the control feed. Water and solid food (treated or untreated) were provided ad libitum for a 2-wk period. Excreta collection from treated and untreated birds was conducted 1 and 2 wk after the beginning of the experiment to test the possible effects of time from the beginning of poultry treatment on fly mortality. For this purpose, an aluminum tray was placed below each cage the day before collection to ensure that feces were produced in the preceding 24 h.

Experiment 4. This experiment followed the same design of experiment 3 and pursued the same objective, but employed hens instead of broilers. In this case the *B. laterosporus* spore suspension was mixed into chicken solid feed (feed-additive method) as previously described to obtain the following range of concentrations that were administered to hens: 10^9, 10^{10} spores/g of diet. The collection of excreta produced by treated

and untreated birds was conducted as described in experiment 3.

Each experiment contained different treatments with 4 replications of 1 bird each.

After collection, all manure samples from different feeding experiments were carried to the laboratory for analysis and storage at −20°C until use in bioassays with flies.

B. laterosporus Quantification in Manure

For *B. laterosporus* residues analysis (cfu), subsamples of chicken manure (1 g) from each bird and collection day were vortex-mixed with 10 mL of PBS buffer at pH 7.4 before being serially diluted. An aliquot (100 μL) of each dilution was then plated in triplicate on LB agar using a sterile glass spreader. *Brevibacillus laterosporus* colony counts were made after 24 to 36 h incubation at 30°C, and their identification was confirmed by morphological observations under a phase microscope.

House Fly Bioassays

Insects used in this study were provided by the rearing facilities of the Dipartimento di Agraria of the University of Sassari (Italy). Fly rearing techniques and conditions are described in Ruiu et al. (2006).

The ability of *B. laterosporus* spores to suppress the development of young house fly larvae in poultry manure was assessed through 2 kinds of experiments. In the first (direct feces treatment), spores were directly mixed with fresh chicken feces, whereas in the second (feces from treated chickens), feces were collected from birds that consumed bacterial spores orally (oral gavage method) or mixed into food (feed additive method) as previously described. The concept behind the latter experiment type is that orally assumed spores were supposed to pass through the alimentary canal and to cumulate in feces, which would produce a residual insecticidal effect.

All bioassays with flies (experiments 1–4) involved 4 replicated manure samples (each from a different bird) that were assayed in triplicate. Groups of 10 second instar larvae were kept inside a Petri dish (3 cm diameter) and exposed to 2 g of a treated or untreated (control) feces sample. After 5 d incubation at 25°C and 50% RH, larval mortality was recorded. Similarly, groups of 10 newly emerged fly adults were maintained at 25°C inside plastic boxes (10 × 15 × 5 cm) with a transparent cover, where 1 g divided into 5 drops of treated or untreated (control) manure was provided at the beginning of the bioassay. A cotton thread continuously soaked in water was also provided to flies for drinking. Adult mortality was recorded after 5 d.

In total, 4 groups of bioassays were conducted: one with feces directly treated with spores (experiment 1), a second with feces from birds treated with spores via oral gavage (experiment 2), a third with feces from broilers treated with spores via feed (experiment 3), and a fourth with feces from hens treated with spores via feed (experiment 4).

In the case of direct feces treatment (experiment 1), excreta from untreated broilers were thoroughly mixed with different amounts of spore suspensions. The following 7 concentrations of spores per gram of feces were assayed on fly adults to estimate the lethal median concentration ($\mathbf{LC_{50}}$) value: 6×10^8, 3×10^8, 1.5×10^8, 7.5×10^7, 3×10^7, 1.5×10^7, and 0.75×10^7. In the case of larvae, the following concentrations of spores per gram of feces were assayed: 2.4×10^8, 1.6×10^8, 1.2×10^8, 6×10^7, 3×10^7, 1.5×10^7, and 0.75×10^7. These experiments were repeated 3 times employing different batches of feces samples and of flies.

In the case of feces from chickens treated via oral gavage (experiment 2) or feed additive method (experiments 3 and 4), fly bioassays involved manure sampled from differently treated and untreated (control) birds as previously described.

Statistical Analysis

All statistical analyses were performed with the SAS system for Windows, version 8.02 (SAS Institute, 1999) with the significance level set at $\alpha = 0.05$.

Mortality data from bioassays employing direct feces treatment (experiment 1) were analyzed by probit analysis for LC_{50} determination (Finney, 1971). Differences were considered significant when 95% fiducial limits did not overlap.

Data on cfu in experiment 2 with feces from broilers fed a daily dose of 10^9 *B. laterosporus* spores were analyzed using repeated measures ANOVA (PROC MIXED), and means were separated using LSMEANS comparison (adjust = Tukey).

Data on fly mortality in experiment 2 with feces from broilers fed a daily dose of 10^9 *B. laterosporus* spores were analyzed as a one-factor design (ANOVA: time) followed by an LSD test to separate treatment means in each sampling interval.

Data on cfu and fly mortality in experiments 3 and 4 with feces from broilers or hens fed a treated diet were analyzed by the GLM of ANOVA (2 factor design: concentration and time), and means were separated by least squares means comparison.

RESULTS

Direct treatment of chicken manure through *B. laterosporus* spores mixing (experiment 1) proved to be effective in causing adult fly death and in suppressing immature development. A maximum mortality (mean ± SEM) of 97.5 ± 3.2% and 98.3 ± 2.1% was achieved with concentrations of 6×10^8 and 2.4×10^8 spores/g manure, for adults and larvae, respectively. As expected, these effects were concentration dependent with an

Table 1. Median lethal concentration for *Musca domestica* adults and larvae fed on poultry feces mixed with *Brevibacillus laterosporus* spores

Insect stage	n[1]	Slope ± SEM	LC_{50} (95% FL)[2]	χ^2	df (*P*-value)
Adults	120	1.98 ± 0.26	1.34 (0.97–1.67)	54.14	1 (<0.0001)
Larvae	120	2.43 ± 0.33	0.61 (0.44–0.76)	54.55	1 (<0.0001)

[1]Number of individuals for each of the 7 concentrations assayed.

[2]Concentration is expressed as number ($\times 10^8$) of spores/g of poultry feces. LC_{50} = lethal median concentration. FL = fiducial limits.

LC_{50} value of 1.34×10^8 and 0.61×10^8 spores/g of feces for adults and larvae, respectively (Table 1).

Fly toxicity was maintained also when insects were reared on feces collected from chickens fed a diet containing adequate concentrations of *B. laterosporus* spores. Feces collected from birds that received bacterial spores via oral gavage for 10 d at a daily dose of 10^9 spores (experiment 2) reached an average cfu content higher than 10^8/g after 3 d of bird treatment. This level significantly decreased after spore administration to broilers was interrupted ($F_{13,143} = 308.75$, $P < 0.0001$; Figure 1). No significant differences among different birds in the cfu values in feces were observed ($F_{13,143} = 3.60$, $P = 0.0656$). This temporal trend was corroborated by fly mortality data of both adults ($F_{14,90} = 52.81$, $P < 0.0001$) and larvae ($F_{14,90} = 73.86$, $P < 0.0001$) that achieved on average 40 to 50% mortality during the treatment interval, followed by a rapid decrease in fly toxicity in the posttreatment period (Table 2).

A higher level of cfu in feces and of fly mortality was achieved in experiments 3 where spores were incorporated in the poultry diet provided to broilers ad libitum at different concentrations (Table 3). On average 11.6×10^8 cfu/g of feces was achieved administering a diet containing 10^{10} bacterial spores/g to broilers. The cfu level in feces was significantly associated to the spore concentration in broilers diet ($F_{2,65} = 1{,}292.43$, $P < 0.0001$), whereas no significant differences emerged between observations conducted after 1 and 2 wk of daily poultry treatment ($F_{1,65} = 0.85$, $P = 0.3599$).

Besides, fly mortality was significantly affected by the concentration of *B. laterosporus* spores in broilers diet either in the case of adults ($F_{3,88} = 287.71$, $P < 0.0001$) and of larvae ($F_{3,88} = 442.09$, $P < 0.0001$) and was significantly different from the control, achieving 79.2% in the case of adults and 88.8% in the case of larvae, when poultry diet contained 10^{10} spores/g. On the other side, no significant differences between different manure collection days (1 and 2 wk after treated diet administration to broilers) were noticed for adults ($F_{1,88} = 0.28$, $P = 0.5963$) and larvae ($F_{1,88} = 2.44$, $P = 0.1221$). Table 3 reports the average fly mortality considering as a factor the bacterial concentration in the poultry diet.

Similarly, in experiment 4, *B. laterosporus* cfu levels in feces were significantly related to hens fed additive concentrations ($F_{1,42} = 1{,}146.22$, $P < 0.0001$), as were the lethal effects recorded on fly adults ($F_{2,65} = 339.15$, $P < 0.0001$) and larvae ($F_{1,65} = 756.23$, $P < 0.0001$). Also in this case, no differences between feces collected in different days (1 and 2 wk during hens treatment period) were noticed for cfu ($F_{1,42} = 3.97$, $P = 0.0530$), adult ($F_{1,65} = 0.78$, $P = 0.3791$), and larval ($F_{1,65} = 0.02$, $P = 0.8815$) mortality. Table 4 reports the average fly mortality considering as a factor the bacterial concentration in the poultry diet.

DISCUSSION

The incorporation of *B. laterosporus* spores in poultry manure causes lethal effects on exposed house fly adults and larvae. These effects are concentration dependent as previously demonstrated with experiments involving spore mixing in artificial diets consisting of wheat bran, milk powder, benzoic acid, and water (Ruiu et al., 2007b). As expected, larvae were more susceptible than adults that showed a 2-fold median lethal concentration. On the other hand, mortality data are comparable to those observed in experiments with cow manure treated with similar concentrations of *B. laterosporus* spores (Ruiu et al., 2008).

As a result of poultry feces analysis, orally consumed *B. laterosporus* spores proved to be able to go through the whole alimentary canal of birds and to be excreted in a concentration correlated to the amount ingested. This includes the capability of spores of this bacterial

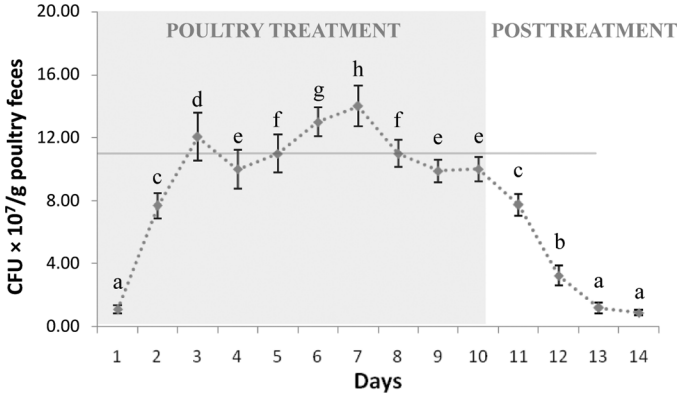

Figure 1. *Brevibacillus laterosporus* cfu (mean ± SD) in feces collected from broilers fed a daily dose of 10^9 spores. Shading indicates the period (10 d) where spores were fed daily to broilers; then they were fed only untreated feed. The horizontal line indicates the average cfu from d 3 to 10 (during the poultry treatment period). Different letters (a–h) indicate a statistically significant difference among means (ANOVA PROC Mixed, $P < 0.05$).

Table 2. Mortality (means ± SEM) of *Musca domestica* (adults and larvae) exposed for 5 d to feces collected from broilers fed for 10 d a dose of 10^9 *Brevibacillus laterosporus* spores

Poultry treatment days	cfu × 10^7/g of feces[1]	Mortality (%)	
		Adults	Larvae
Treatment			
0	—	0.8 ± 0.83[a]	1.7 ± 1.67[a]
1	1.1 ± 0.07[a]	2.5 ± 1.31[ab]	4.2 ± 2.29[ab]
2	7.7 ± 0.23[c]	7.5 ± 2.50[abc]	9.2 ± 3.58[bc]
3	12.1 ± 0.44[d]	32.5 ± 2.50[d]	36.7 ± 4.32[d]
4	10.0 ± 0.35[e]	40.8 ± 2.88[ef]	51.7 ± 2.41[ef]
5	11.0 ± 0.34[f]	37.5 ± 1.79[de]	48.8 ± 4.53[fg]
6	13.0 ± 0.27[d]	39.2 ± 2.29[de]	50.8 ± 2.88[efg]
7	14.0 ± 0.37[g]	44.2 ± 3.36[efg]	55.8 ± 2.60[e]
8	11.0 ± 0.24[f]	46.7 ± 2.56[fg]	50.8 ± 1.93[efg]
9	9.9 ± 0.21[e]	39.2 ± 2.60[de]	52.5 ± 2.79[ef]
10	10.0 ± 0.22[e]	48.3 ± 2.71[g]	44.2 ± 2.88[g]
Posttreatment			
1	7.7 ± 0.20[c]	25.0 ± 2.89[h]	33.3 ± 1.88[d]
2	3.2 ± 0.18[b]	10.8 ± 3.13[c]	15.8 ± 2.88[c]
3	1.2 ± 0.10[a]	7.5 ± 2.50[abc]	11.7 ± 2.71[c]
4	0.9 ± 0.05[a]	9.2 ± 2.29[bc]	1.7 ± 1.12[a]

[a–h]Means within a column with different superscript letters differ (ANOVA PROC Mixed for cfu, 1-way ANOVA for mortality, followed by least significant difference test, $P < 0.05$).

[1]Spore viability (cfu) in feces samples was determined by plating serial dilutions of the original suspensions on Luria-Bertani agar.

species to survive the acidic stomach juice and to reach plentifully the intestine. In our experiments, the concentration of vital spores in feces, expressed as cfu, were on average 10 times less concentrated than in the diet administered to birds, which would presume the death of some spores during the path toward the intestine or their partial retention in the alimentary tract. After all, in the case of other *B. laterosporus* strains, the administration of appropriate spore concentrations as a feed additive can produce probiotic effects in mammals as a result of their survival in the intestine (Hong et al., 2005). In the case of poultry, an orally administered daily dose of about 2 million cfu significantly improves feed conversion and weight gain (Porubcan, 2003). Furthermore, the property of *B. laterosporus* spores to survive extreme acidic environments is corroborated by the results of ultrastructural observations in the gut of flies where after being orally administered to insects, intact spores were detected in the endo-perithrophic space of the hind-midgut, proving they went through the acid mid-midgut (pH 3.1; Ruiu et al., 2012).

The relative potential of poultry feed additives to control fly larvae in manure was highlighted by Loomis et al. (1968) who achieved significant results in experiments where hens were fed different concentrations of the organophosphate insecticide coumaphos. Effectiveness in fly larvae control was also observed employing insect growth regulators such as cyromazine (Axtell and Edwards, 1983) or other insecticides such as methoprene (Breeden et al., 1981) as feed additives whose residues cumulate in feces.

Significant toxicity of livestock feces against flies was previously observed feeding animals with *Bacillus* spp. treated diets (Briggs, 1960). Borgatti and Guyer (1963) determined the insecticidal effects of *B. thuringiensis*-based commercial formulations in droppings from the Japanese quail *Coturnix coturnix japonica* Temminck and Schlegel. In that case commercial formulations had a concentration with an order of magnitude corresponding to 10^{10} spores/g and were administered to birds ad libitum for 70 d at a rate around 7 g/lb (3.15 g/kg) of food. A significant effectiveness calculated in comparison to an untreated control was achieved with a treatment of 14 g/lb (6.3 g/kg) of food corresponding to 4.6×10^{10} spores consumed by a quail pair per day. In line with our results, the same authors highlighted a

Table 3. Mortality (means ± SEM) of *Musca domestica* (adults and larvae) exposed to feces from broilers fed for 2 wk a diet containing different concentrations of *Brevibacillus laterosporus* spores

No. of spores/g of poultry diet	cfu × 10^8/g of feces[1]	Adult mortality	Larval mortality
10^{10}	11.6 ± 0.29[a]	79.2 ± 2.25[a]	88.8 ± 1.84[a]
10^9	1.7 ± 0.07[b]	52.9 ± 2.21[b]	56.3 ± 1.89[b]
10^8	0.4 ± 0.06[c]	24.6 ± 1.99[c]	32.9 ± 1.85[c]
Control	—	2.9 ± 1.12[d]	2.5 ± 1.24[d]

[a–d]Means within a column with different superscript letters differ (GLM PROC of ANOVA, followed by least significant difference test, $P < 0.05$).

[1]Spore viability (cfu) in feces samples was determined by plating serial dilutions of the original suspensions on Luria-Bertani agar.

Table 4. Mortality (means ± SEM) of *Musca domestica* (adults and larvae) exposed to feces from hens fed for 2 wk a diet containing different concentrations of *Brevibacillus laterosporus* spores

No. of spores/g of poultry diet	cfu × 10^8/g of feces[1]	Adult mortality	Larval mortality
10^{10}	9.6 ± 0.20[a]	82.9 ± 1.85[a]	90.0 ± 1.59[a]
10^9	2.5 ± 0.13[b]	47.5 ± 3.09[b]	57.1 ± 1.85[b]
Control	—	2.1 ± 1.04[c]	2.5 ± 1.24[c]

[a–c]Means within a column with different superscript letters differ (GLM PROC of ANOVA, followed by LSD test, $P < 0.05$).

[1]Spore viability (cfu) in feces samples was determined by plating serial dilutions of the original suspensions on LB agar.

concentration-dependent effect. In addition, they conducted observations on the fate of ingested spore preparations in the digestive tract of birds, corroborating our observations on a minimal spore retention, which would explain the rapid drop in fly toxicity if feces are collected from birds after the administration of the treated diet is replaced with the untreated one. By contrast, in the case of cyromazine, a higher posttreatment retention of fly toxicity in manure from lying hens was noticed after feeding the chemical to the birds from hatch to 20 wk of age (Brake et al., 1991).

In the course of time, several investigators considered the use of *B. thuringiensis* as a feed additive for different animals obtaining a good efficacy in suppressing house fly development in feces. Besides poultry species, these studies included experiments with cattle (Dunn, 1960), steers (Harvey and Brethour, 1960), and pigs (Ramisz et al., 1994). The fly control effectiveness in feces was attributed to the presence of bacterial spores and insecticidal toxins. Among the latter, the crystal proteins (Cry) contained in parasporal bodies produced by the bacterium and acting in the midgut of susceptible insects (Pigott and Ellar, 2007). However, past studies involving experiments with flies and microbials, for the most part employed commercial formulations based on *B. thuringiensis* var. *thuringiensis*, whose toxicity against the house fly was generally related to the production of β-exotoxin or thuringiensin (Hall and Arakawa, 1959; Harvey and Brethour, 1960), an insecticidal heat-stable adenine-nucleotide analogous, whose use in agriculture is now prohibited (Levinson et al., 1990; Vega and Kaya, 2012).

More recently, experiments with commercial formulations containing *B. thuringiensis* var. *israelensis* as a feed additive for the control of house fly larvae in caged-poultry manure were conducted (Mwamburi et al., 2011). Also in this case, larval susceptibility was concentration dependent and increased with the duration of exposure. A 90% reduction in larval density in field conditions was achieved after feeding poultries for 4 wk with a diet containing 10 g of *B. thuringiensis* var. *israelensis*/kg. Insect mortality was associated to the bacterial production of parasporal bodies containing insecticidal toxins belonging to Crystal (Cry) and Cytolitic (Cyt) protein families (Bravo et al., 2007).

In the case of *B. laterosporus*, the toxicity against flies is a toxin-mediated process reminiscent of the mechanism of action of *B. thuringiensis* δ-endotoxins, and spores with their canoe-shaped parasporal body have been identified as the main fly toxic fraction (Ruiu et al., 2007b). The insecticidal potential of liquid formulations containing *B. laterosporus* spores was previously demonstrated in the field context drawing the attention to the need of an application method ensuring the uniform mixing of spores with manure (Ruiu et al., 2008, 2011). The main concern with liquid formulations sprinkled on the surface is their difficulty reaching fly larvae in the deeper manure mass. In addition, given a continuous animal excretion, bioinsecticidal applications should be periodically repeated on fresh manure. On the other hand, a uniform and constant mixing of bacterial spores with manure mass might be achieved through their administration as a poultry feed additive.

Based on our results, it is apparent that the majority of spores ingested by poultry species, pass through the whole alimentary canal, and cumulate in feces making them toxic to both fly adults and larvae. Notably, the insecticidal power against both insect stages represent an added value in the potential to control this pest species. In addition to the lethal effects, *B. laterosporus* spores ingested at sublethal dosages causes the immature development slowing down, and the reduction in adult longevity and reproductive potential, thus contributing to a further suppression of fly population density (Ruiu et al., 2006).

The fate of insecticidal residues in livestock environments is another noteworthy aspect, considering that the manure management in the farm may include its reuse in soil for amendment purposes (Ghosh et al., 2004). Nevertheless, the presence of *B. laterosporus* residues in manure from treated animals can contribute to increase soil fertility, as supported by the recently highlighted fertilization properties associated to this bacterium that is also commercially available in soil amendments (Porubcan, 2005). On the other side, soil represents a natural habitat for *B. laterosporus* that produces highly nematotoxic proteases (Lian et al., 2007) and antimicrobial compounds (Zhao et al., 2012), thus contributing to plant root protection from various pathogens and parasites (Ruiu, 2013).

Besides, the general safety of this entomopathogenic species for the main nontarget muscoid fly parasitoids (Ruiu et al., 2007a) supports its compatibility with integrated pest management programs in the livestock environment.

The introduction of multifunctional microbial control agents such as *B. laterosporus* in animal production systems may provide an additional contribution to the prospect of a more integrated approach to farming. A wider picture arising from the presented concepts includes the use of feed additives producing beneficial (probiotic) effects on animals and at the same time indirectly contributing to contain insect pests developing in manure and plant parasites in amended soil. Current and future research findings toward this direction will support an eco-sustainable vision of the farm considered as a whole.

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