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Poultry: *Gallus gallus domesticus* L. 'Chicken'

Evaluation of bacterial treatments (*Bacillus* spp.) for control of immature house flies, 2024

Alec C. Gerry*, Sydney Wilson, Hannah H. Chu, Karla Lemus, Amy C. Murillo

Department of Entomology, University of California at Riverside, Riverside, CA 92521, USA *Corresponding author, email: alec.gerry@ucr.edu

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House fly | *Musca domestica* L.

Laboratory and semi-field trials were conducted using 2 multi-strain blends of *Bacillus* spp. bacteria being evaluated by a collaborating company for control of immature house flies. These blends are experimental and lack a specific product name. Bacillus blend #1 is a 3-strain blend of *B. amyloliquefaciens* and *B. licheniformis*. Bacillus blend #2 is a blend of the same bacterial strains as blend #1, but also includes an additional strain of *B. amyloliquefaciens*. Bacteria in the genus *Bacillus* are rod-shaped, gram-positive bacteria with bacterial species examined in this study used as biotic amendments to poultry feces/litter to support composting and reduce ammonia production. We were interested in testing whether these bacteria might also reduce the development of immature house flies in treated poultry litter. Bacterial blends were formulated by the collaborating company with a maltodextrin-based carrier to form a shelf-stable powder that can be dissolved into water for application to fly development media. Additional treatments evaluated were a strain of bacteria (*Bacillus thuringiensis* [unspecified subspecies]) obtained by the collaborating company and similarly formulated with the maltodextrin carrier and 2 negative check treatments (nothing added; maltodextrin carrier only), for a total of 5 separate treatments. In the laboratory trial, treatments were applied to standard house fly-rearing media composed of bran, alfalfa, dry milk, and yeast (Zahn and Gerry, 2018). In the semi-field trial, treatments were applied to poultry litter which was a mix of dry chicken feces and Timothy hay. Chicken feces was acquired from chickens housed at UC Riverside. The chicken feces were dried for >1 month on a covered drying pad and were free of fly larvae at the time of use in the semi-field trial. For both the laboratory and semi-field trials, dry-rearing media (laboratory media or poultry litter) was prepared in sufficient quantity for all 5 treatments. Dry media was homogenized, and 1 kg of dry media was placed into each of 5 10.7 L rearing pans (Rubbermaid Dishpan 2951, Atlanta, GA). To prepare each treatment, 1 g of a bacterial treatment (or carrier only) was mixed into 1.5 L of cold tap water in a 2 L glass beaker with continuous stirring using a stir bar and magnetic plate stirrer. Each aqueous treatment (or water alone for the nothing added check treatment) was slowly added to a labeled house fly-rearing pan while manually mixing the treatment into the media using a wooden stirrer. A small sample (~2 g) of each treatment media was subsequently removed to determine moisture content (65–69% for laboratory media or 62–72% for poultry litter across all replicates). Approximately 1,000 house fly eggs (0.5 mL eggs measured volumetrically in water) from a recently colonized (F3–4) field strain of house flies collected from a southern California dairy (San Jacinto, CA) were added to each pan. Pans were covered with mesh bags and placed in a laboratory room at 26 °C and 50% relative humidity (RH) (lab trial) or in a covered location within an empty experimental poultry house at the University of California at Riverside (semi-field trial). In the semi-field trial, rearing pans with immature house flies were protected from direct sun and precipitation but were otherwise exposed to natural outdoor environmental conditions (daily means: 24–29 °C, 41–64% RH). In the semi-field trial, rearing pans were also protected from foraging ants by placing them on tables with each table leg inserted into a 5 G bucket containing soapy water. In both the lab and semi-field trials, when larvae had pupated (6–8 days after adding eggs to pans), the rearing pans were covered with an emergence trap containing a small amount of commercial fly bait (Quikstrike fly bait, Central Life Sciences, Schaumburg, IL) to kill adult flies as they emerged from the rearing pan. Emergence traps were checked daily, and dead adult flies were removed from the trap and counted by sex. Treatments were replicated on three separate dates for both the laboratory and semi-field trials.

Adult flies were recovered in similar numbers and with a similar sex ratio from all treatments, including negative check treatments, within each trial (Table 1). Immature fly development times were also similar among all treatments (data not shown). Data analyses were performed separately for the laboratory and semi-field trials using 2-factor analysis of variance (PROC GLM in SAS v. 9.4) with Treatment and Replicate as independent variables and with total emerged flies (both sexes combined) as the dependent variable. There were no significant differences among treatments in the laboratory trial ($P = 0.54$) or the semi-field trial ($P = 0.61$). While there was a significant difference in total adult flies produced among replicates in the laboratory trial ($P = 0.0002$) with replicate #1 having fewer total

Table 1. Fly production (emerged adults) by treatment applied during laboratory and semi-field trials (mean and SE of 3 replicates per treatment)

Trial	Treatment	# Emerged adults		% Female
		Mean	SE	
Laboratory (lab media)	Control: no treatment	951	156	50.1
	Control: carrier only	1,070	384	49.5
	Bacillus sp. #1	1,251	428	50.9
	Bacillus sp. #2	981	412	48.8
	Bacillus thuringiensis	1,117	294	51.8
Semi-field (poultry litter)	Control: no treatment	763	48	53.1
	Control: carrier only	599	172	49.6
	Bacillus sp. #1	927	273	50.0
	Bacillus sp. #2	857	46	50.4
	Bacillus thuringiensis	633	253	47.0

flies than other replicates, there was no interaction among treatments and replicates ($P > 0.05$) indicating this effect was not related to treatments. There were no differences in total flies emerged among replicates in the semi-field trial ($P = 0.18$).

Overall, fewer flies were recovered from the rearing pans in the semi-field trial relative to the laboratory trial even though all rearing pans in both trials received the same number of eggs. The lower adult production in the semi-field trial may be due to very hot outdoor conditions during Jul 2024 when the semi-field trial was performed (max air temperature reached 35–38 °C) or to generally lower productivity of the poultry litter used in the semi-field trial relative to the standard media used in the laboratory trial. No deformed or unemerged pupae were observed in any treatment.

Because each aqueous treatment was thoroughly mixed into rearing pans rather than applied to the surface of each pan, lack of treatment effect relative to the negative check treatments is not due to poor penetration of the treatment into the media where immature flies develop, as has been reported for other studies where treatments were applied only to the surface of the development substrate. Our laboratory media is formulated with yeast providing flies with another microbial food source that might compensate for lethal effects of the bacterial treatments. However, the similar lack of differences among treatments when flies were reared on poultry litter in the semi-field trial suggests that yeast supplementation in the laboratory media was not responsible for the lack of treatment effect. We did not test persistence of the bacterial products in the rearing pans for either the laboratory or semi-field trials and therefore cannot exclude that treatment bacteria applied to the rearing media did not survive long, perhaps outcompeted by other microbes present in the media. Future trials might increase the bacterial concentration applied to rearing media and assess bacterial persistence within pans to address these questions. (This research was supported by industry gifts of funding and bacterial products for testing.)

There were no significant differences among treatments for either the laboratory or semi-field trails ($P > 0.05$) using 2-way analysis of variance with treatment and replicate as independent variables. Raw mean data for all 3 replicates of each treatment are shown.