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Effects of Lime Hydrate on the Growth and Development of Darkling Beetle, *Alphitobius diaperinus*

D. W. Watson[1], S. S. Denning[1], L. Zurek[2], S. M. Stringham[1] and J. Elliott[3]
[1]Department of Entomology, North Carolina State University, Raleigh, NC 27695, USA
[2]Department of Entomology, Kansas State University, Manhattan, KS 66506, USA
[3]Chemical Lime, P. O. Box 985004, Ft. Worth, TX 76185-5004, USA
E-mail: Wes_Watson@ncsu.edu

Abstract: The addition of hydrated lime to poultry litter to control insects and pathogens has a history of support. We examined the effects of hydrated lime litter treatments on the darkling beetle, *Alphitobius diaperinus* and the fungal pathogen *Aspergillus*. Hydrated lime application rates were calculated as poultry house equivalents; 22.6, 45.4, 56.7, 90.7 kg per 93 m^2 (50, 100, 125 and 200 lbs per 1,000 ft^2), ground limestone; 22.6 kg per 93 m^2 and an untreated control. Among treatment groups, mortality was significantly higher for larvae than for adult beetles. Hydrated lime at the highest rate (90.7 kg per 93 m^2) produced 59.1 and 24.6% mortality for larvae and adults, respectively. Limestone did not increase beetle mortality. Darkling beetle mortality was moisture dependent with greatest larval mortality (100%) observed at 90.7 kg/93 m^2 and 68% moisture while adult mortality was 58.8%. Effects of lime hydrate on the number of bacterial and *Aspergillus* colony forming units (CFUs) in treated litter was inconclusive. The impact of hydrated lime on beetles and perhaps pathogens in litter is likely the direct effect of increased pH, however the numbers of beetles, and fungal or bacterial CFUs may increase as pH levels become more neutral.

Key words: Darkling beetle, lesser mealworm, poultry pest management, poultry disease, *Aspergillus*

Introduction

The darkling beetle, *Alphitobius diaperinus* Panzer, is a common pest of chicken and turkey production. The darkling beetle larva, commonly called the lesser mealworm, may undergo 6-10 molts before pupation. The adult beetle is dark brown to black and about 7 mm in length. Darkling beetle development is temperature dependent, requiring 29 days from egg to adult at 35 °C and 134 days at 20 °C (Rueda and Axtell, 1996). Adult beetles may live several months to a year and a female beetle produces about 3.81 eggs/day during its lifetime (Preiss and Davidson, 1968).

All life stages of the darkling beetle are found in poultry litter and manure, where they feed on manure, litter, meal, dead birds, and other insects, including one another (Leschen and Steelman, 1988). Pre-pupating larvae cause direct damage by tunneling into the foam-core insulation used in poultry house construction (Vaughan *et al*., 1984; Geden and Axtell, 1987). Also the darkling beetle has been incriminated in the transmission of several disease agents. These include viral diseases such as Newcastle disease, avian influenza, infectious bursal disease, Marek's disease, fowl pox, *Reovirus, Rotavirus,* and *Coronavirus* (De la Casa *et al*., 1973, 1976; Despins *et al*., 1994; McAllister *et al*., 1995; Watson *et al*., 2000). Other disease causing agents carried by darkling beetles include *Salmonella, Escherichia coli*, and numerous other known pathogens (McAllister *et al*., 1994, 1996). The natural movement and dispersal of beetles increase the potential for the spread of disease on and between farms. Occasionally, nuisance issues arise as darkling beetles invade residences following land-application of manure and litter fertilizer (Miller, 1997).

The darkling beetle is the predominant litter inhabiting insect species of North Carolina turkey houses and its management is important for efficient production (Axtell, 1994; Rueda and Axtell, 1997). The typical NC turkey house has an earthen floor covered with 5-18 cm of pine shavings, peanut hulls, or another absorbent material (Axtell, 1999). After depopulation, turkey litter may remain in the house for the next flock or be removed and used as organic fertilizer. The practice of frequent clean out reduces beetle populations within poultry houses, but remains a potential source of dispersal and re-infestation.

The use of lime hydrate (calcium hydroxide) in agriculture has a long history. Livestock and poultry producers would whitewash barns and buildings to reduce interior temperatures, and added lime to manure and litter to reduce odors. Evidence suggests hydrated lime treatments may reduce or repel insects (Barata *et al*., 1992; Boucher and Adams, 1993) and pathogens (Munoz *et al*., 1995; Stanush *et al*., 2000). However, dolomitic limestone and hydrated lime did not significantly reduce densities of Japanese beetles six weeks following a single treatment in Massachusetts' soils (Vittum, 1984). In contrast, significant initial

reductions in bacterial counts were observed in cattle bedding following the addition of lime (Hogan *et al*., 1999).

Clearly, the benefits of hydrated lime for insect and pathogen control are not well defined. Recognized as a desiccant, lime hydrate was recommended to treat outdoor latrines, dead animals and other situations requiring rapid drying. Hydrated lime absorbs excess moisture and promotes drying, causing a rapid rise in pH ($\geq$12.0). In the process hydrated lime combines with water in the substrate, releasing ammonia and creating a hostile environment for insects and pathogens. Our objectives were threefold: Objective 1: Determine mortality or growth suppression of larval and adult darkling beetles exposed to formulations of hydrated lime added to moistened poultry manure and litter substrates. Objective 2: Determine the residual effects of hydrated lime for activity against larval and adult darkling beetles in a turkey house. Objective 3: Evaluate antimicrobial activity of hydrated lime treatments of turkey litter under laboratory conditions.

Materials And Methods

Adult and larval darkling beetles were collected from the NCSU laboratory colony. Adult and second instar beetles were separated into 6 groups of 20 individuals each. Turkey litter was frozen to kill any insects or mites. Thawed litter was transferred to large bins and water was thoroughly mixed into the samples. Hydrated lime treatments were added to each bin and thoroughly mixed. Rates of hydrated lime were calculated as poultry house equivalents applied at 22.6, 45.4, 56.7, and 90.7 kg per 93 m^2 (50, 100, 125 and 200 lbs per 1,000 ft^2), ground limestone applied at 22.6 kg per 93 m^2, and an untreated control. Treated litter was dispensed to 500 ml plastic drink cups in 350 cc quantities each. Treatments were replicated three times (60 beetles per treatment, and 6 treatments). Beetles were added to each cup and a plastic lid fixed in place. A small opening (0.5 cm diameter) was made in each lid to allow air exchange. Cups were held at 32 ^oC (85 ^oF). One additional replicate was set up without the addition of beetles to monitor pH during the experiment. Experiments were repeated five times. Litter moisture levels of 48, 58, 61 and 68% were established by adding water to known litter volumes. Beetle mortality was examined after 7 days. Means were calculated on the number of surviving beetles per treatment and analyzed with GLM ANOVA (Minitab, 1997), then converted to percent mortality. Percent mortality was corrected for control mortality by using Abbott's formula (Abbott ,1925).

Litter and distilled deionized water were mixed at a rate of 1 to 10 for pH measurements. Samples were measured using a Corning pH meter (Corning, NY) immediately upon mixing hydrated lime and moist litter, then at 24 and 96-hour intervals.

Litter samples taken from four lime-treated turkey houses were examined for moisture, number of bacterial and the fungal (*Aspergillus*) CFUs. One house was treated with ground limestone at 22.6 kg per 93 m^2 (50 pounds per 1,000 ft^2), while the remaining three houses received 22.6, 45.4, and 56.7 kg per 93 m^2 (50, 100 or 125 pounds/1000 ft^2) of hydrated lime, respectively. Litter samples were taken prior to application and again after lime had been applied and tilled into the used litter pack to compare moisture and pH. The house was repopulated with turkey poults one week later. Four months following the initial treatment, these houses were sampled for darkling beetles using 10 tube traps per house (Safrit and Axtell, 1984). Adult and larval darkling beetles were enumerated one week following trap placement.

Bacterial and fungal (*Aspergillus*) CFUs were quantified using standard plate count methods. CFUs in the lime-treated and untreated turkey bedding were enumerated by suspending three 10g litter samples in Erlenmeyer flasks with 95 ml sterile potassium phosphate buffer (3.0 mM) and 50 µl of Tween 80 (polyoxyethylene sorbitan monooleate). Samples were placed on a rotary shaker (250 rpm) for 1 hr and then serially diluted in phosphate buffer to 10^{-7}. To select for bacteria, 100 µl aliquots from each dilution were spread in triplicate on Trypticase Soy Broth Agar [25] with cycloheximidine (33.5 mg/l, w/v). Similarly, aliquots were spread in triplicate on the Czapek's medium (Difco, 1984) with 6.5% NaCl (Payne *et al*., 1988) to select for *Aspergillus*. Plates were incubated aerobically at room temperature (25 ^oC). The enumeration of CFUs was conducted after 4 days of incubation for bacteria, and 7 days of incubation for fungi. The final count was adjusted to 1 gram of bedding dry weight.

Results and Discussion

Addition of hydrated lime to litter samples increased mortality among adult and larval darkling beetles in the laboratory (Fig. 1). Mean percent mortality of the untreated control was 11.5 ± 0.59 and 9.5 ± 0.45, for larvae and adults, respectively. Treatment mortality figures presented were corrected for control mortality (Abbott, 1925). Among treatment groups, mortality was significantly higher for larvae than for adult beetles ($P \leq 0.001$, $F = 26.41$, $df = 87$). Hydrated lime at rates of 22.6, 45.4, 56.7, and 90.7 kg per 93 m^2 induced 10.2, 40.2, 59.4 and 59.1% larval mortality, respectively ($P \leq 0.001$, $F = 13.38$, $df = 5$). Percent mortality for adult beetles was 1.1, 3.7, 11.4, and 24.6 at rates of 22.6, 45.4, 56.7, and 90.7 kg per 93 m^2, respectively. Limestone did not impact beetle survival, with mortality rates of 2.9 and 1.9% for adults and larvae, respectively.

Darkling beetle mortality was moisture dependent. Larval mortality was 47.0 and 66.5% at lime treatment rates of 45.4 and 56.7 kg/93 m^2 and 48%, respectively

Table 1: Mean percent mortality[1] effects of litter moisture on the efficacy of limestone and lime hydrate against darkling beetle larvae

Treatment	Percent Litter Moisture				Significance		
	48	58	61	68	P =	F =	df
22.6 kg[2] Limestone	7.0±0.33	3.5±0.33	23.5±0.91	8.5±0.88	0.026	5.61	11
22.6 kg Hydrate	7.0±0.66	7.0±0.67	31.6±1.50	25.0±1.15	0.107	2.90	11
45.4 kg Hydrate	47.0±2.60	13.5±0.88	38.5±1.26	98.3±0.33	0.001	40.18	11
56.7 kg Hydrate	66.5±0.66	15.0±0.01	70.0±2.00	98.3±0.33	0.001	2.24	11
90.7 kg Hydrate	No data	8.5±0.33	73.3±2.04	100.0±0.00	0.001	9.85	9

[1]Mean percent mortality and SE were corrected from control mortality (Abbott, 1925).
[2]Crushed limestone and lime hydrate were applied to litter as poultry house equivalents: 22.6, 45.4, 56.7 and 90.7 kg per 93 m^2.

Table 2: Mean percent mortality[1] effects of litter moisture on the efficacy of limestone and lime hydrate against darkling beetle adults

Treatment	Percent Litter Moisture				Significance		
	48	58	61	68	P =	F =	df
22.6 kg[2] Limestone	6.7 ± 0.33	6.6± 1.33	15.0± 0.73	16.6 ± 1.20	0.428	0.93	11
22.6 kg Hydrate	6.7 ± 0.33	6.7 ± 1.33	12.5 ± 0.80	13.5 ± 1.20	0.684	0.40	11
45.4 kg Hydrate	21.6 ± 2.85	8.3 ± 0.88	11.7 ± 0.76	10.0 ± 0.01	0.832	0.19	11
56.7 kg Hydrate	26.6 ± 1.45	8.3 ± 0.88	16.6 ± 1.15	30.0 ± 1.53	0.162	16.33	11
90.7 kg Hydrate	No data	13.5 ± 0.66	24.1 ± 1.40	65.0 ± 2.00	0.002	19.58	9

[1]Mean percent mortality and SE were corrected from control mortality (Abbott, 1925).
[2]Crushed limestone and lime hydrate were applied to litter as poultry house equivalents: 22.6, 45.4, 56.7 and 90.7 kg per 93 m^2.

(Table 1). Adult beetle mortality was 21.6 and 26.6 % at 45.4 and 56.7 kg/93 m^2 (Table 2). We added a rate of 90.7 kg/93 m^2 to the study to extend the lime hydrate treatment range. Beetle mortality was low in all treatments at litter moisture level of 58% (Tables 1 and 2). These unexplained results were inconsistent with subsequent experiments. Increasing litter moisture to 61% increased larval mortalities to 62.9 and 67.0% at 56.7 and 90.7 kg./93 m^2, respectively (Table 1). Greatest larval mortality (>98%) was observed at 68% litter moisture and lime application rates of 45.4, 56.7, 90.7 kg per 93 m^2. Adult mortality was 65.0% at 90.7 kg./93 m^2 and 68% moisture (Table 2).

The impact of hydrated lime on beetle larvae in wet litter is likely a direct effect of increased pH (Table 3). The highest rate of hydrated lime in litter at 68% moisture produced and sustained pH >11 for at least 96 hours. Lesser rates produced lower pH, which could not be sustained for more than 24 hours. Notably, ground limestone had little or no effect on litter pH.

Under field conditions there was little evidence of a long-term residual effect of lime treatments on darkling beetle densities (Fig. 2). Four months following treatment larval and adult beetle densities were lowest (12.5 ± 3.24 and 57.8 ± 7.02, respectively) in the house treated with 23 kg of hydrated lime/93 m^2. Darkling beetle densities were significantly greater in houses that received the highest rates of lime hydrate ($P \leq 0.01$, $F = 3.94$, $df = 79$, $P \leq 0.01$, $F = 3.60$, $df = 79$, Fig. 2).

A detectable increase in ammonia occurred immediately following the application of the lime to the litter. Ammonia levels had dissipated by bird placement 1-week later. Although the litter was not particularly wet at the time of treatment, hydrated lime reduced litter moisture between 4 and 9% during the 4-month interval between pre and post-treatment evaluations (Fig. 3). Pretreatment moisture levels in houses 1 to 4 were 52.8, 48.4, 49.5, and 44.9 percent, respectively. One week post-treatment, moisture levels were reduced to 28.05, 36.7, 45.7 and 35.5 percent but generally increased during the 4-month period to 55.0, 44.5, 40.5 and 39.6 percent in houses 1 to 4, respectively ($P = 0.63$, $F = 0.53$, $df = 31$). Pretreatment litter samples had a neutral pH, 6.15, 6.28, 5.62, and 6.90, for houses 1 to 4, respectively. Post-treatment pH levels increased to > 8.0 in all houses, regardless of treatment for 6-weeks.

Numbers of *Aspergillus* and bacteria CFUs were reduced at 45 kg rate of lime hydrate (Table 4). However, pre and post treatment reductions in fungal and bacterial CFUs were not significant ($P = 0.73$, $T = - 0.35$, $df = 4$, $P = 0.82$, $T = 0.24$, $df = 4$), respectively. Observed reductions in microorganisms were thought to be attributable to the pH shift (Miskimmin *et al*., 1995). The effects of pH on fungal and bacterial growth may

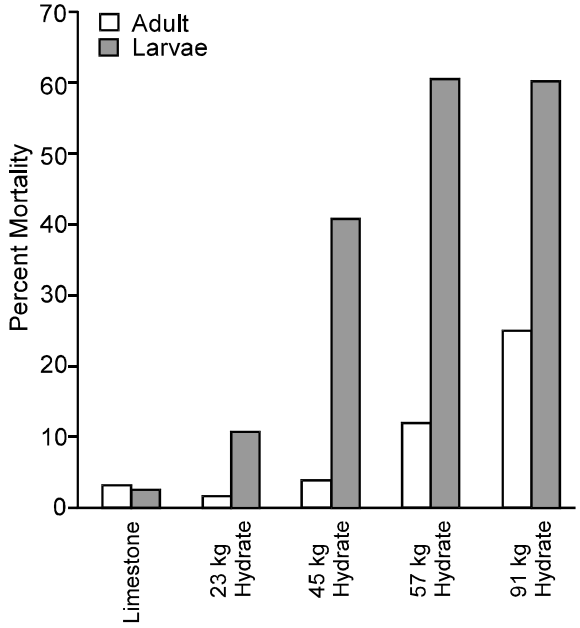

Fig. 1: Effect of limestone and lime hydrate litter treatments on adult and larval darkling beetle survival.

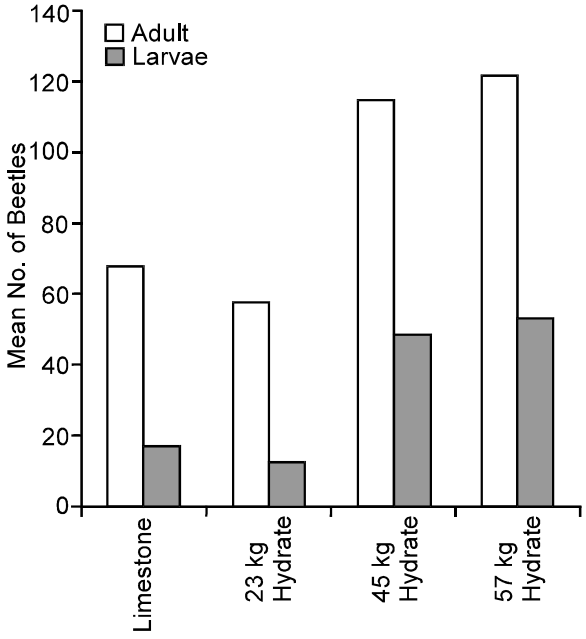

Fig. 2: Mean number of adult and larval darkling beetles collected from tube traps four months following litter treatments with limestone and lime hydrate.

have been greater if litter moisture was near 68% (Table 3). Since such pH shifts are temporary the number of fungal and bacterial CFU may increase, as pH levels become more neutral. Hogan *et al*. (1999) observed

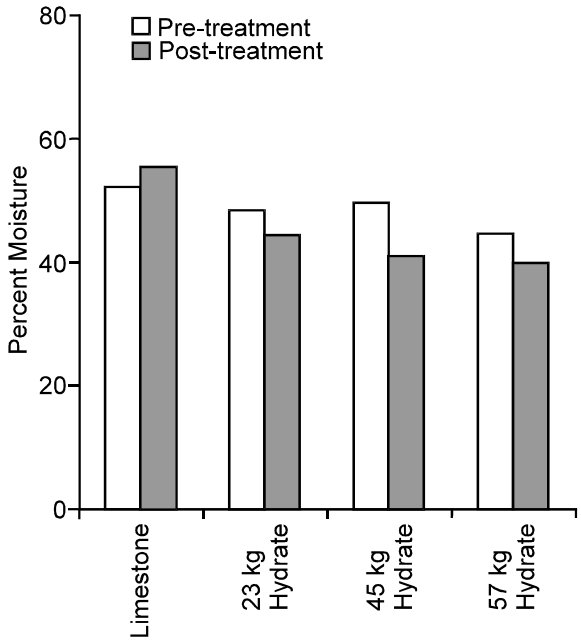

Fig. 3: Percent moisture of litter treated with limestone and three rates of lime hydrate.

Table 3: Hydrogen ion concentration (pH) of limestone and lime hydrate treated litter (68% moisture) from <1 to 96 hours post-treatment

Treatment[1]	Hours posttreatment		
	<1	24	96
Control	7.86	8.10	8.44
22.6 Limestone	7.90	8.14	8.56
22.6 Hydrate	10.48	8.44	8.64
45.4 Hydrate	11.62	10.36	8.50
56.7 Hydrate	11.86	10.78	9.40
90.7 Hydrate	12.08	11.74	11.26

[1]Crushed limestone and lime hydrate were applied to litter as poultry house equivalents: 22.6, 45.4, 56.7 and 90.7 kg per 93 m^2.

similar conditions in hydrated lime treated dairy manure in which bacterial CFU decreased initially then rebounded 2 and 6 days following treatment. Stanush *et al*. (2000) significantly reduced *Salmonella enteritidis* and other bacteria in lime treated litter. Although we did not observe a significant change in the number of CFUs in this study, pH shifts were expected to inhibit the growth of microorganisms, albeit temporarily.

In summary the addition of hydrated lime to poultry litter increased mortality of larval and adult darkling beetles in laboratory tests. Larval mortality was greater than that of adults. Mortality effects of lime hydrate were dose and moisture dependent over a range of 22.6 to 90.7 kg per 93 m^2 (50 to 200 lbs per 1,000 ft^2). Greatest larval mortality (100%) was observed at 90.7 kg per 93 m^2 and

Table 4: Colony forming units (CFUs) of *Aspergillus* and bacteria after lime treatments were applied to litter used litter in four turkey houses

	Aspergillus		Bacteria	
Treatment (kg/m2)	Pre	Post	Pre	Post
Limestone 22.6	$6.36x10^2$	$3.33x10^4$	$0.57x10^8$	$6.0x10^7$
Hydrated 22.6	$1.94x10^2$	$4.50x10^2$	$1.36x10^8$	$6.67x10^7$
Hydrated 45.4	$2.56x10^3$	$4.22x10^2$	$1.23x10^8$	$4.39x10^7$
Hydrated 56.7	0	$4.97x10^2$	$1.11x10^8$	$2.15x10^8$

[1]Crushed limestone and lime hydrate were applied to litter as poultry house equivalents: 22.6, 45.4, 56.7 and 90.7 kg per 93 m^2.

68% moisture while adult mortality was 58.8%. The impact of hydrated lime on beetles in litter is probably a direct effect of increased pH. We observed a limited, though not significant, reduction in numbers of bacterial and *Aspergillus* CFUs in litter from treated turkey houses where moisture levels did not exceed 53%. Although high moisture levels were not found in the field study, occasional wet spots in the poultry house could be effectively treated with hydrated lime to dry the area. Given adequate litter moisture levels, the numbers of beetles, fungal and bacterial CFUs may decline initially then increase as pH become more neutral. Although hydrated lime killed darkling beetle larvae under laboratory conditions, its use as an aid in the control of darkling beetles has not been fully evaluated in the field.

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