

## Timing Diatomaceous Earth-Filled Dustbox Use for Management of Northern Fowl Mites (Acari: Macronyssidae) in Cage-Free Poultry Systems

Amy C. Murillo<sup>1</sup> and Bradley A. Mullens

Department of Entomology, University of California, Riverside, CA 92521 (alock001@ucr.edu; bradley.mullens@ucr.edu), and

<sup>1</sup>Corresponding author, e-mail: alock001@ucr.edu

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### Abstract

Northern fowl mite management on conventionally caged birds relies on synthetic pesticide sprays to wet the vent. Cage-free chickens cannot be effectively treated this way, and pesticide use is restricted in organic production. Dustbathing behavior is encouraged in newer production systems for increased hen welfare. Diatomaceous earth (DE) is an approved organic insecticide that can be mixed with sand in dustboxes, suppressing mites but not excluding them, and potentially allowing development of mite immunity. We tested two hypotheses: 1) that DE-filled dustboxes placed before northern fowl mite introduction (prophylactic use) prevents mite populations from reaching economically damaging thresholds, and 2) that bird exposure to low mite numbers allows for protective hen immunity to develop and suppress mites after dustboxes are removed. We also tested if different beak trimming techniques (a commercial practice) affect mite growth. Mites were introduced to birds after dustboxes were made available. Average mite densities in flocks remained below damaging levels while dustboxes were available. Average mite populations rebounded after dustbox removal (even though DE persisted in the environment) regardless of the timing of removal. Mite densities on birds where a traditional hot-blade beak trimming technique was used (trial 1) were high. Mite densities in trial 2, where a newer precision infra-red trimming was used, were lower. The newer infra-red trimming method resulted in nearly intact beaks, which were better for mite control by bird grooming behaviors. The combination of early dustbox use and infra-red beak trimming should allow producers to avoid most mite damage in cage-free flocks.

**Key words:** Northern fowl mite, dustbathing, integrated pest management, organic, cultural control

The northern fowl mite, *Ornithonyssus sylviarum* (Canestrini & Fanzago), is the most common ectoparasite of poultry in the United States and causes significant economic losses in egg-laying hens (Axtell and Arends 1990, Mullens et al. 2009). Typical outbreaks occur in flocks in the first egg production cycle, and mites are introduced by infested pullets, contaminated personnel or equipment, wild birds, or carry-over mites from a previous flock (Kells and Surgeoner 1996, Axtell 1999, Chen and Mullens 2008). Once mites are established on a property, they can be very difficult to eliminate and may become a predictable problem in every subsequent new flock.

The economic impact of northern fowl mites can be serious for producers, as northern fowl mite infestations cause reduced egg production, reduced egg weights, and reduced feed conversion efficiency (Mullens et al. 2009). In a recent study, northern fowl mite infestations reduced feed conversion efficiency by up to 17% (Murillo et al. 2016), an economic burden to producers who spend ~70% of their budget on poultry feed (Bell and Weaver 2002). Most of this economic impact is likely linked to physiological costs related to host immune responses. Numerous studies have

demonstrated hen-immune reactions in the form of mite-specific antibodies (DeVaney and Ziprin 1980, Burg et al. 1988, Minnifield et al. 1993) and vent skin inflammation (Owen et al. 2009). White leghorn hens infested with mites for 4–10 wk experience large reductions in mite numbers, caused by the host immune system (Owen et al. 2008, Mullens et al. 2009). However, data on economic impact in commercial egg layer flocks (Mullens et al. 2009) suggest that mite densities below a visual score of 3 (approximately 100 mites/bird) do not cause the distinct level of economic damage linked to higher-level infestations. Keeping mite densities below this threshold should mitigate or eliminate economic damage caused by northern fowl mite on egg-layers, while still allowing enough low-level mite exposure for birds to develop an immune response.

Many birds, including chickens, perform dustbathing behavior, which maintains feather integrity by removing excess lipids (Olsson and Keeling 2005). Birds prefer to dustbathe in finer materials like sand, as opposed to coarser substrates such as wood chips or AstroTurf (Olsson and Keeling 2005, Scholtz et al. 2010, Vezzoli et al. 2015). Martin and Mullens (2012) showed that heavily infested birds dustbathing in a sand-diatomaceous earth (DE) mixture

reduced mite numbers from an average score of 6 (~5,000 mites) to an average score of 3 (~100 mites) within one week. However, those dustboxes were only used reactively to control mites, after populations were high. Dustboxes with substrate and DE have the potential to be used to prevent mite establishment or buildup early in the life of a flock and keep mites below economically damaging levels. Low-level mite exposure could also allow naïve hens the opportunity to acquire adaptive mite immunity while avoiding economic damage. Dustboxes are advantageous because they are inexpensive and easy to set-up and maintain in cage-free housing systems. In addition, DE is listed for unrestricted use by the Organic Materials Research Institute (OMRI), so this control method could be implemented in conventional or organic cage-free production.

Commercial birds are routinely beak-trimmed as very young chicks to reduce feather pecking, cannibalism, and feed waste (Hester and Shea-Moore 2003, Mertens et al. 2009). Birds used in trials 1 and 2 were sourced from the same local producer. However, in the second year, they had converted from using a traditional electric hot-blade trimmer to using a newer infra-red (IR) trimming technology (Dennis and Cheng 2010). While not a designed part of the initial study, the different beak conditions between trials 1 and 2 provided an additional point of comparison, as beak condition affects ectoparasite loads on hens (Chen et al. 2011). The IR method resulted in substantial beak regrowth (Fig. 1) by the time the hens reached adulthood. Many beaks in trial 2 were almost fully intact (able to close cleanly with mandible overlap at the tips), which we predicted may impact mite population growth.

In this study, we hypothesized: 1) DE-filled dustboxes placed before northern fowl mite introduction (prophylactic use) prevent mite populations from reaching economically damaging thresholds; 2) hen exposure to low mite numbers, while dustboxes are present, allows for protective hen immunity to develop and mite populations will be suppressed (evidence of immunity) after dustboxes are removed; and 3) the newer IR beak trimming method allows birds to groom more efficiently, thus negatively impacting mite population growth. We allowed birds access to DE mixed with sand in dustboxes prior to experimental mite exposure to test if mite establishment or population growth would be disrupted. We removed

dustboxes at 8 and 12 wk to determine if mites would rebound, or if sufficient immunity had developed.

## Materials and Methods

### Chickens

ISA (Institut de Sélection Animale) brown female chickens (18–19-wk-old) were housed in cage-free poultry houses at the University of California Riverside Agricultural Operations under UC Riverside Institutional Animal Care and Use Protocol A-20150009. Two structures (3.8 by 5.8 m) were divided into two separate sections, each section equipped with water dispensers, feed troughs, and nest boxes. These met or exceeded U.S. standards for cage-free production (United Egg Producers 2010). Each of the four sections, hereafter called a house, held 18 birds (a flock) within an area 1.5 by 3.1 m. Straw bedding approximately 5–10 cm in depth was added to each house at the beginning of the study and was not removed until the study concluded. Additional straw was added halfway through the study. Lights were kept on a 16:8 (L:D) h cycle. Hardware cloth screens (6-mm openings) allowed air flow into the houses while excluding wild birds and rodents. Roof sprinklers moderated high temperatures. Each hen was uniquely marked with colored leg bands for individual identification.

### Mites

Northern fowl mites were aspirated using pipettes from source hens maintained at UCR Agricultural Operations and were used to inoculate experimental birds (Martin and Mullens 2012). Once a week for the duration of the study, vent feathers of all treatment hens were visually scored (by ACM) for level of mite infestation (mite populations). The scoring system used was as follows: 1 = 1–10, 2 = 11–50, 3 = 51–100, 4 = 101–500, 5 = 501–1000, 6 = 1001–10,000, and 7 = >10,000 (Arthur and Axtell 1983).

### Dustboxes

Black PVC plastic cement-mixing bins (Plasgad Plastic Products ACS Ltd Kibbutz, Gadot, Upper Galilee, IL) 60 by 90 by 9 cm in depth were used for dustboxes (Fig. 2). Food-grade DE (Perma-Guard Inc., Bountiful, UT, USA) was mixed with washed play sand



**Fig. 1.** Trial 1 birds (left) were beak-trimmed as young chicks by a commercial breeder using a hot-blade trimmer. Note blunt beak and minimal regrowth in adult. Trial 2 birds (right) were beak-trimmed as chicks using an infra-red (IR) trimmer. Note substantial beak regrowth to a sharp tip and some upper and lower mandible overlap in adult. A more intact beak allows birds to groom more effectively, thus negatively affecting ectoparasite populations.

(Premium Play Sand; Quikrete Companies, Inc., Atlanta, GA, USA). A 9:1 ratio by weight of sand (16.2 kg) to dust (1.8 kg) was mixed together, which yielded a depth of ~5 cm of material in each box. Dustboxes were recharged with ~900 g DE on a weekly basis and sand was added as needed to keep the materials at a depth of ~5 cm.

A scale from 0–3 (adapted from Martin and Mullens 2012) was used to estimate bird dustbox use by examining DE in the feathers for each bird weekly (always by ACM). Chickens will dustbathe on average every other day for ~30 min (Vestergaard 1982), which is frequent enough that any dust from bathing in the past 1–2 d would be detected. Scores were: 0 = no visible dust; 1 = slight dust on exterior feathers; 2 = dust distinctly apparent on parted/disturbed feathers in vent area; 3 = airborne dust plume obvious when feathers disturbed. A score of 0 or 1 indicated no dustbox use for that week (no dustbathing), while a score of 2 or 3 indicated dustbox use (dustbathing).



Fig. 2. Black plastic bins contained 5–10 cm of diatomaceous earth and sand for hens to dustbathe.

### Dustbox Study

At the start of the study (week 1), each of the four flocks was given access to a dustbox prophylactically, before mites were experimentally introduced. One week later (week 2), all birds were each inoculated with 20–30 adult mites (Fig. 3). All birds were reinfested at week 3 with 20–30 additional adult mites each to mimic continued exposure. Mite scores and dustbox use were monitored weekly for individual birds in each of the four flocks.

Six weeks after the first mite inoculation (week 8), after visually scoring each bird for northern fowl mite and dustbox use, dustboxes were removed from two of the four flocks (flocks 1 and 2 in each trial). All birds were scored visually for northern fowl mite, and flocks 3 and 4 were scored weekly for dustbox use. Four weeks later (week 12), dustboxes were removed from the two remaining flocks (3 and 4; after visually scoring birds for northern fowl mite and dustbox use). All birds were visually examined weekly for northern fowl mite for 4 additional weeks, for a total length of 16 wk per trial. Two trials (January–April 2014 and December–March 2015) were conducted.

### Beak Trimming and Grooming Efficiency

To evaluate the efficiency of grooming and mite reduction in the IR-trimmed birds, 24 mite-infested birds from trial 2 were randomly selected at the end of the second trial period (week 16). Birds were placed individually into battery cages with no opportunity to dustbathe. After one week, all birds were scored for mite infestation, paired by similar mite scores, and then were randomly assigned to either a control (no beak bit) or treatment (beak bit) group. Treatment birds were fitted with adult-sized “ChickNBits” (Decker Mfg. Co., Keokuk, IA, USA) beak bits. The bits are used commercially to decrease feather pecking by preventing the bird’s beak from closing completely, but do not affect eating or drinking behaviors (Fig. 4). This additionally interferes with ectoparasite grooming by birds (Clayton and Tompkins 1995). Mite populations were scored on control and beak-bitted birds for 4 additional weeks.

Trial	Flock	Week															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1 & 2	Dustboxes added	Mites added	Mites and dustbox use scored (m + d)	m + d →					m + d, dustbox removed	Mites scored →						
3 & 4		Dustboxes added	Mites added	Mites and dustbox use scored (m + d)	m + d →				m + d	→		m + d, dustbox removed	Mites scored →				
2	1 & 2	Dustboxes added	Mites added	Mites and dustbox use scored (m + d)	m + d →				m + d, dustbox removed	Mites scored →							
3 & 4		Dustboxes added	Mites added	Mites and dustbox use scored (m + d)	m + d →				m + d	→		m + d, dustbox removed	Mites scored →				

Fig. 3. In trials 1 and 2, dustboxes were added 1 wk (week 1) before mites were introduced. Mite populations and dustbox use were scored (m + d) each week. In flocks 1 and 2 (each trial), dustboxes were removed at week 8 (after m + d). Flock 3 and 4 (each trial) dustboxes were removed at week 12 (after m + d). Shaded regions indicate when dustboxes were absent. After dustbox removal, mite populations only were scored weekly.



## Statistical Analyses

Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, 2010, v. 9.3). Proc means were used to generate averages and standard errors of mite scores.

Regression slopes (mite score versus time) were used to examine mite population trends (increasing, stable, or decreasing) while the dustbox was present or absent in a flock. For trial 1, flock 1 and flock 2 regression slopes were based on weeks 1–8 (dustbox present) and weeks 9–16 (dustbox absent). Trial 1 flock 3 and flock 4



**Figure 4.** Bird fitted experimentally with beak bit to impair ability to groom at the end of trial 2.

“present” slopes were analyzed for weeks 1–12 and “absent” slopes for weeks 13–16. In trial 2, flock 1 and flock 2 were combined for “present” and “absent” regression analyses because of overall low mite scores. Trial 2 flocks 3 and 4 were not included in this analysis because of very low mite populations.

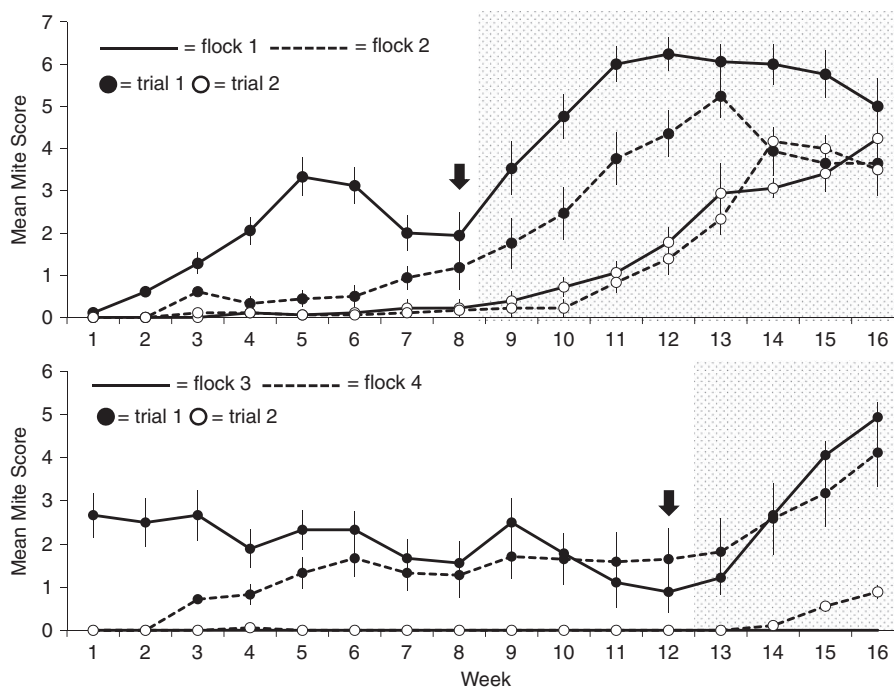
Mixed-model repeated-measures analyses were used to examine general trends of the main effect of dustbox use on mite scores while dustboxes were present. Scores of 0 or 1 indicated birds that did not use the dustbox (non-bathers), while scores of 2 or 3 indicated birds that were bathers for a given week. Bird was the fixed effect and week was the repeated measure. A comprehensive model was used initially, then analyses were separated by trial and flock. Flocks 1 and 2 were evaluated during weeks 1–8 only, while flocks 3 and 4 were evaluated during weeks 1–12 only. Two-sample *t*-tests were then used to examine mean mite scores in trial 1 between groups (non-bather vs bathers) for each week while the dustbox was present. Trial 2 was not included in this analysis because mite populations while dustboxes were present were too low to compare bathers and non-bathers.

## Results

In trial 1, one bird in each of flocks 1, 2, and 4, and in trial 2, one bird in flock 1 was removed (injury or death) during the course of the study and was not replaced.

### Trial 1

Average mite populations varied by flock (Fig. 5). Birds in flocks 1 ( $n=2/18$ ) and 3 ( $n=18/18$ ) were accidentally exposed to mites sometime shortly prior to intentional experimental infestation (week 2), but mean mite densities were still low and below a score of 3. Regression slopes for flocks 2 and 4 showed slow but positive mite population growth (slopes 0.16,  $P < 0.01$  and 0.15,  $P < 0.001$ , respectively) while dustboxes were present. Slopes were steeper and distinctly positive after dustboxes were removed in those flocks



**Fig. 5.** Mite population trends (means  $\pm$  SE) in flocks where dustboxes were removed at week 8 (top, flocks 1 and 2) or week 12 (bottom, flocks 3 and 4) as indicated by arrow. Shaded areas indicate mite counts in weeks when dustboxes were absent. Trial 1 birds (filled circle) generally had higher mite scores than trial 2 birds (open circle).

(slopes 0.24,  $P < 0.05$  and 0.75,  $P < 0.05$ , respectively). Mite numbers dropped in flock 3 (early mite exposure; slope  $-0.13$ ,  $P < 0.01$ ) while the dustbox was present compared with steep mite increases when it was removed (slope 1.26,  $P < 0.0001$ ). Flock 1 had an increased rate of mite growth when the dustbox was available (slope 0.40,  $P < 0.0001$ ), but mite numbers remained very low overall (scores below 2). Mites increased further when the box was removed (slope 0.18,  $P < 0.05$ ) and eventually reached average scores of about 6 in flock 1.

In all four trial 1 flocks, mite scores increased distinctly with the removal of the dustboxes, even after infestation periods of 9–12 wk (Fig. 5).

While dustboxes were available to birds in flocks 1, 2, and 3, hens that were scored as non-bathers averaged significantly higher mite scores than bathers (flock 1: bathers =  $0.75 \pm 0.16$ , non-bathers =  $2.38 \pm 0.22$ ;  $F = 39.45$ ;  $df = 1, 6$ ;  $P = 0.0008$ ; flock 2: bathers =  $0.18 \pm 0.06$ , non-bathers =  $0.67 \pm 0.18$ ;  $F = 32.62$ ;  $df = 1, 13$ ;  $P < 0.0001$ ; flock 3: bathers =  $1.31 \pm 0.16$ , non-bathers =  $2.86 \pm 0.23$ ;  $F = 5.71$ ;  $df = 1, 16$ ;  $P = 0.030$ ). For flock 4, there was no significant difference between bathers and non-bathers while dustboxes were present (bathers =  $0.33 \pm 0.08$ , non-bathers =  $1.73 \pm 0.21$ ,  $F = 0.82$ ;  $df = 1, 16$ ;  $P = 0.38$ ).

Average mite scores between these two groups in flocks 1 and 2 (Fig. 6) and flocks 3 and 4 (Fig. 7) were compared by week using  $t$ -tests. In general, bathers had lower weekly mite populations than non-bathers. The number of bathers varied from week to week, however, but only one individual hen in each trial ( $n = 1/72$ ) never showed evidence of dustbathing while dustboxes were present.

### Trial 2

Average mite populations in trial 2 were overall much lower than in trial 1 ( $F = 39.93$ ;  $df = 1, 142$ ;  $P < 0.0001$ ; Fig. 5). There was a slight positive relationship between mite growth and time for flocks 1 and 2 (combined) while dustboxes were present (slope 0.03;  $P < 0.05$ ), but mite scores were very low and averaged less than 1 (fewer than 10

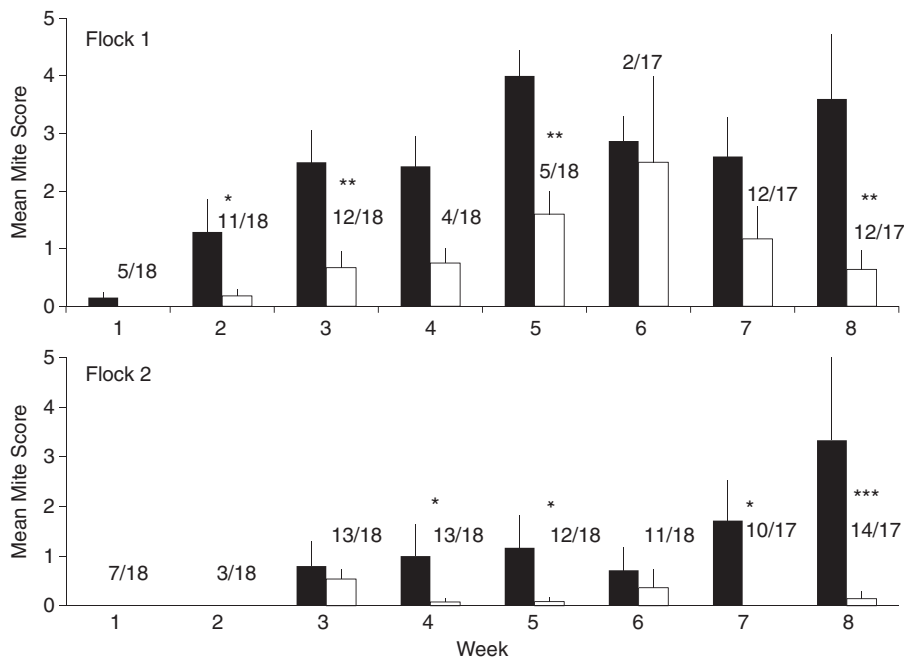
mites/hen). As in trial 1, mite scores increased rapidly when the dustboxes were removed (slope 0.55;  $P < 0.0001$ ). Mite populations eventually reached scores of 3 to 4 in flocks 1 and 2 and scores of about 1 in flock 4, whereas mites failed to establish in flock 3 (Fig. 5).

### Beak Trimming and Grooming Efficiency

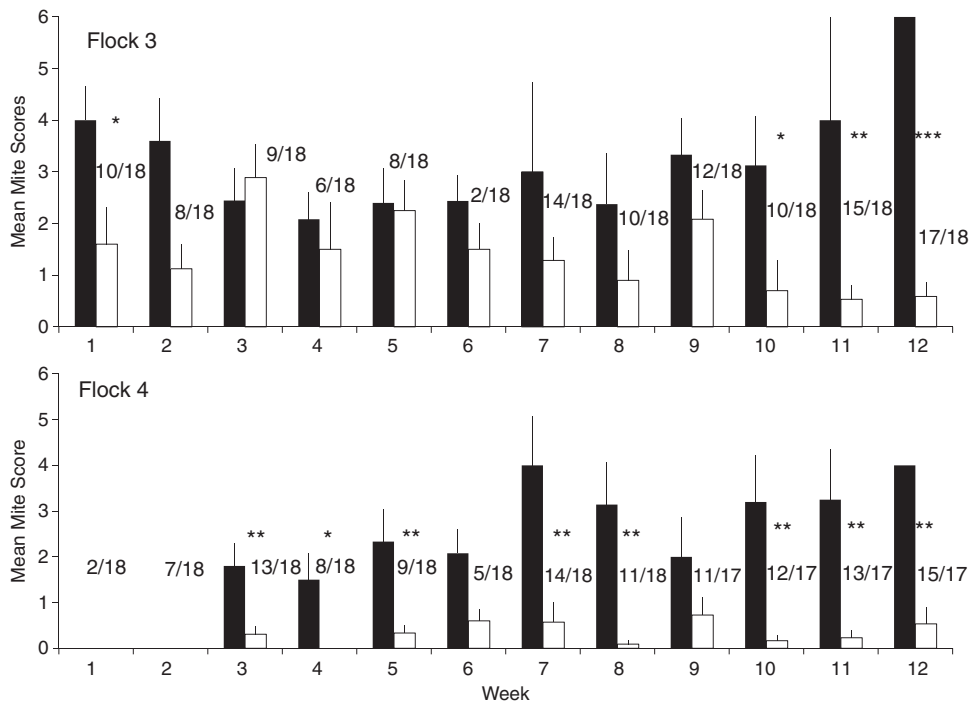
Trial 2 mite numbers were lower (Fig. 5) compared with numbers on hens with permanently and uniformly blunt tips (trial 1). Once the second dustbox trial was complete, mite populations on birds equipped with beak bits were compared with mites on unaltered birds (Fig. 8). Mite densities between the control and treatment groups were comparable before beak bits were added (week 1;  $T = 0.86$ ;  $df = 22$ ;  $P = 0.40$ ). Mite populations on beak-bitted birds grew quickly, and after one week, there was a significant difference in overall mite scores between the two groups of birds (Fig. 8). Beak-bitted hens harbored mite numbers approximately twice as high relative to hens that did not receive bits.

### Discussion

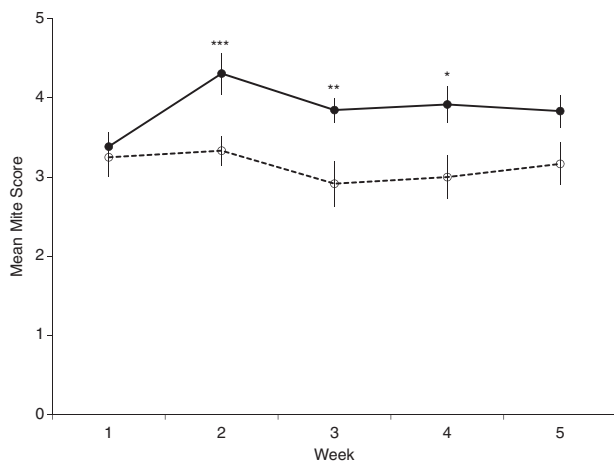
In general, mite populations grew very slowly while dustboxes were present in both trials compared with when dustboxes were absent. In trial 1, two flocks (1 and 3) were accidentally exposed to low mite numbers before birds were experimentally infested. In flock 3, mite populations declined over time when dustboxes were present, likely because mite populations were a little higher before the study began. In this flock, the dustbox addition was no longer prophylactic, but still early enough to keep mite numbers low. Trial 2 mite populations were very low in flocks 1, 2, and 4, and completely absent in flock 3 while dustboxes were present. Once dustboxes were removed from each house, mite populations increased in all of the chicken flocks, with the exception of flock 3, which never had detectable mite populations. In flock 3, mites never established, even with repeated, intentional mite introduction.



**Fig. 6.** Mean ( $\pm$  SE) mite scores of birds scored as non-dustbathers (black) and dustbathers (white) in flocks 1 and 2 (top and bottom, respectively). The number of hens dustbathing within each week are shown out of the total number of birds. Within a week, significant mite differences between groups are indicated with asterisks: \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$ .



**Fig. 7.** Trial 1 mean ( $\pm$  SE) mite scores of non-dustbathers (black) and dustbathers (white) in flocks 3 and 4 (top and bottom, respectively). The number of hens dustbathing within each week are shown out of the total number of birds. Within a week, significant mite means between groups are indicated with asterisks: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .



**Fig. 8.** Mean ( $\pm$  SE) mite scores of birds with beak bits (solid line) compared with unaltered controls. Beak bits were added to birds after week 1 mite scores. Asterisks indicate: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .

Trial 2 birds harbored low mite numbers, but when birds were beak-bitted, we saw increased mite growth in just 1 wk. The beak condition of hens, due to the different trimming techniques, was an important factor in keeping mite populations low. Birds will groom and kill ectoparasites, a behavior that is distinctly impaired by hot-blade beak trimming (Chen et al. 2011). The IR technology, that was used on trial 2 birds by the producer, allowed beaks to regrow enough for birds to groom better. However, this behavior alone was not sufficient to keep mite populations suppressed once dustboxes were removed in flocks 1, 2, and 4.

Despite early accidental mite exposure in two flocks during trial 1, average mite scores in all houses for both trials were held at or below the target economic threshold of a visual score of 3, or  $\sim 100$

mites per bird, while dustboxes were present. Previous work on the economic impact of northern fowl mites in commercial egg layer flocks (Mullens et al. 2009) suggests that mite densities below a visual score of 3 do not result in significant economic effects (e.g. decreased feed conversion efficiency). Reduced mite numbers also cause fewer poultry worker concerns with mite irritation and safety associated with the application of traditional pesticides. When dustboxes were deployed before mites were experimentally introduced, mites were maintained well below this threshold for damage. This was true even with variable dustbox use by individual birds. Non-dustbathing individuals generally harbored higher mite numbers in a given week, and probably served as sources of mite inoculum for the other birds in the group. Still, because most individual birds showed evidence of dustbox use at some point during the trial period, the overall number of mites available in that house to infest different birds was suppressed.

Despite prolonged bird exposure to low levels of mites while dustboxes were available over periods of 8 or 12 wk, we observed no evidence for the development of adaptive immunity to mites by hens. We did not measure immune effectors (e.g. antibodies) directly, but could infer activity via mite population trends. In white leghorn hens that experience initial high mite infestation, this is sufficient time to see clear suppression of mites by immune responses, with reductions of over  $100\times$  in mite densities relative to peak numbers 3–6 wk after exposure (Mullens et al. 2009, Owen et al. 2009). As mites blood-feed, they cause skin inflammation and thickening of the epidermis, primarily in the vent region. This inflammation physically blocks mites from successfully blood-feeding and is linked to the major histocompatibility complex genes of white leghorn layers (Owen et al. 2008, 2009). ISA brown hens are a hybrid cross between a white leghorn and Rhode Island red, and immune responses to ectoparasite loads have not yet been examined in this strain, which is preferred for use in cage-free egg production. ISA brown

hens may not mount as strong an immune response to northern fowl mite as white leghorns, or it may not occur over the same time frame. It is also possible that low mite numbers, as observed when dustboxes were present, were not sufficient to trigger a strong immune response. Martin and Mullens (2012) noted that northern fowl mite densities on ISA Brown or Hyline Brown hens would drop over time, but could remain high for 2–3 mo after initial northern fowl mite exposure on some birds. Burg et al. (1988) noted that Fayoumi hens injected earlier with crude mite extracts developed lower mite numbers after live northern fowl mite exposure, but that trend did not appear with White Rock strain hens, again suggesting strain differences in immune response to northern fowl mite. Further work is needed to assess the effects of ectoparasites such as northern fowl mite on host immune responses and economic factors on breeds used in cage-free systems, such as ISA brown.

The DE in dustboxes was recharged weekly to ensure fresh material for birds to use. Sand was replaced as needed, which varied by house. In many cases, birds would try to dustbathe as soon as fresh DE was provided. Dustbathing is an innate, complex behavior that has benefits both for bird feather condition and well-being (Appleby et al. 1993, Olsson and Keeling 2005). Dustbathing is also likely a socially facilitated behavior (Lundberg and Keeling 2003), and variation observed in overall dustbathing could be dependent on which individuals make up a flock. Social hierarchy is known to effect resource use (Shimmura et al. 2008) and might be expected to result in variable dustbox use among flock members. Dominant individuals may spend more time dustbathing or try to defend the dustbox resource from less dominant individuals.

Dustbathing could be adaptive, in that dustbathing in certain natural substrates such as clay has ectoparasite-suppression benefits (Martin and Mullens 2012), but there is no evidence to date that dustbathing is actually driven by parasite loads. Vezzoli et al. (2015) examined the number and duration of dustbathing bouts of northern fowl mite-infested birds held in furnished cages, and relative intensity of mite populations did not influence dustbathing behavior of birds provided with AstroTurf or sand. More work in this area is desirable to determine if frequency or duration of dustbathing in finer substrates is influenced by type or intensity of ectoparasite load (mites or other ectoparasites such as lice). In addition, an increased drive to dustbathe could reflect social hierarchy position if this is a highly desirable resource. At any rate, understanding the basic factors that influence dustbathing is important to best exploit this behavior for ectoparasite suppression at the flock level. For example, failure of certain hens to dustbathe in DE allows them to harbor high mite loads, possibly for long periods, and serve as mite reservoirs in a flock.

DE was kicked out of the dustboxes while the birds dustbathed, and dust was observed in the bedding of each house outside the boxes. After the dustboxes were removed, DE still persisted and was visible in the environment. Birds were observed dustbathing in the straw bedding when dustboxes were not available. However, this level of residual DE was not enough to keep mite scores low after the boxes were removed. The same result was seen earlier in hens dustbathing in fine litter particles plus residual DE or kaolin clay (Martin and Mullens 2012). When birds perform dustbathing behavior, they move the substrate up and into their feathers to remove excess lipids (Olsson and Keeling 2005). Sand alone, or substrates with similar particle sizes, are suitable for dustbathing, but do not reduce mite numbers (Martin and Mullens 2012, Vezzoli et al. 2015). DE is much finer and acts as a desiccant against arthropods, including mites (Quarles 1992, Cook et al. 2008, Kilpinen and Stenbergh 2009). It is probable that the main substrate (sand here)

acts as a carrier to facilitate the movement of DE into the feathers, where DE then acts on the mites by abrasion and adsorption of surface wax, killing mites via desiccation (Ebeling 1971, Quarles 1992). The concentrated availability of DE with the fine sand substrate in a dedicated dustbox is important to achieving mite suppression. Incidental exposure of birds and their mites to DE in the environment is not enough.

One of the more interesting aspects of this study is the differences in mite populations between the two trials. Trial 2 birds maintained very low mite numbers while dustboxes were available. Yet mites were able to increase once dustboxes were removed, reaching average mite scores greater than 3 (100 mites/bird) in just 6 wk (flocks 1 and 2). Trial 2 birds were beak-trimmed using IR-beak-trimming technology. IR-trimmed birds fitted later with beak bits quickly doubled their mite numbers compared with unaltered controls. This helps implicate effective hen grooming behavior (with a mostly intact beak) as an important factor in suppressing mite populations. The ability of bit-free IR-beak-trimmed birds to groom helped control mite populations in trial 2, relative to birds with blunter beaks in trial 1. It is interesting, however, that mite populations did not stay low after dustboxes were removed in trial 2 with the improved grooming ability of IR-beak-trimmed birds. As producers improve welfare and move toward IR-beak-trimming or eliminate beak trimming altogether (with docile breeds), we can expect to see a better level of ectoparasite control through host-grooming. Our results indicate improved grooming by IR-trimmed hens may not be enough, by itself, to keep mites dependably below damaging levels. The combination of a cultural control tactic (beak trimming technique) and a mechanical control tactic (DE dustboxes) worked best to keep mite scores below economically damaging levels. This is beneficial to bird welfare as well.

In the current study we were able to mimic production densities but were unable to reproduce commercial flock sizes. Commercial cage-free flocks can have many thousands of birds. More on-farm work is required to elucidate actual placement, size, and number of dustboxes per flock to achieve effective ectoparasite control. When dustboxes are used prophylactically, northern fowl mite populations on flocks grew slowly and were, on average, at or below ~100 mites per bird, which limits the economic damage caused by mites. Mite populations were even more depressed when birds were beak-trimmed using IR technology. However, mite suppression was not sustained when dustboxes were removed 6 or 10 wk after mites were first introduced to flocks. Using DE in dustboxes also allows birds to exhibit natural dustbathing behaviors, thus increasing animal welfare and decreasing the use of labor-intensive pesticide applications that can be harmful to human, animal, and environmental health.

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