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Use of *Beauveria bassiana* to Control Northern Fowl Mites (*Ornithonyssus sylviarum*) on Roosters in an Agricultural Research Facility

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Treatment of Northern fowl mite (*Ornithonyssus sylviarum*) infestation on poultry in research facilities can be challenging. The mite has a rapid reproductive cycle (egg to adult in 5 to 7 d), and chemical treatments can be toxic to birds, personnel, and the environment. In addition, antimite treatment may interfere with experimental research designs. The current study evaluated the efficacy of topical application of an entomopathogenic fungus, *Beauveria bassiana*, in the treatment of a naturally occurring infestation of Northern fowl mites in pen-housed roosters (*n* = 14; age, 18 mo). Two groups of 7 roosters each were used in 2 experiments: *Beauveria* (30 mL, 2.9×10^{10} spores per bird) compared with water (30 mL, control), and *Beauveria* compared with the common topical organophosphate agent tetrachlorvinphos–dichlorvos (30 mL). We also assessed a higher dose of *Beauveria* (300 mL, 2.9×10^{11} spores per bird) in the 7 birds that were not exposed to tetrachlorvinphos–dichlorvos. *Beauveria* reduced mite levels relative to the control group but did not outperform tetrachlorvinphos–dichlorvos when used at an equal volume and frequency. Increasing the volume and frequency of *Beauveria* application improved outcomes such that visual inspection failed to detect any mites. The results presented here suggest that, when applied in sufficient doses, *Beauveria* effectively reduces mites on poultry and can be an important part of an integrated pest management program. Additional research is needed to document the most effective dose, frequency, and location of *B. bassiana* application to control Northern fowl mites in poultry.

Abbreviation: NFM, Northern fowl mite.

The Northern fowl mite (*Ornithonyssus sylviarum*; NFM) is a hematophagous ectoparasite of chickens (*Gallus gallus domestica*) that poses longstanding and significant challenges to both commercial poultry producers and facilities using poultry in biomedical and agricultural research.

The NFM completes its entire life cycle on the host, although the mite reportedly can survive off an animal for as long as 3 wk[15] and does not need direct contact between birds to disseminate quickly throughout poultry housing.[25] Due to a life cycle that can be as short as 5 d, mite levels can increase rapidly, with effects ranging from immune system changes, decreased body condition, and anemia to death.[20,26] As a result, this arthropod pest poses great concerns regarding the health of affected chickens, data collected by investigators using chickens as a research model, and even the health of poultry producers.

Successful management of poultry flocks involves sound biosecurity practices, which go hand-in-hand with integrated pest management programs that emphasize preventative over control measures. In the case of NFM, when these preventative measures fail, chemical treatment often is attempted. However, such treatments carry their own risks to birds, persons applying the treatment, research data, and the environment.[13,27]

As a result, demand exists among both poultry researchers and poultry producers to identify efficacious treatments that carry fewer risks than do chemical antimiticides. One promising novel treatment is the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin. *Beauveria* is a fungus found in the soil that, when cultivated appropriately, infects various insects and arthropods and may be useful in controlling their levels.[17,28]

The current study explored the effectiveness of a commercially available isolate of *Beauveria* in controlling a naturally occurring NFM infestation in roosters. We hypothesized that this fungus would perform better than water and at least as well as the current standard of care in our facility, a topical organophosphate (RAVAP-EC), in lowering the level of observable NFM on groups of naturally infested roosters.

Materials and Methods

Animals and housing. Animals were maintained in accordance with the recommendations set forth in the *Guide for the Care and Use of Agricultural Animals in Research and Teaching*[12] and under an AAALAC-accredited animal program at the University of Wisconsin-Madison. All experimental procedures were approved by the College of Agricultural and Life Sciences Animal Care and Use Committee. Male (*n* = 14; age, 18 mo) chickens of the Regional Poultry Research Laboratory (RPRL) 7.1 line originating from Michigan State University were used in the current study. The birds were not beak-trimmed and received vaccination against Marek disease virus (gallid herpesvirus 2), Newcastle disease virus, infectious bronchitis virus, avian encephalomyelitis virus, infectious bursal disease, avian reovirus, and fowlpox virus. The facility where the animals were housed is monitored annually for *Salmonella pullorum* by serology, and bimonthly drag swabs of litter are used to monitor for the presence of *Salmonella enteriditis*. The roosters were identified by using leg bands and housed in approximately 12 ft × 6 ft pens on woodchip litter with an automatic water source, ad libitum feed, and several roosts. The pens were maintained on a 12:12-h light:dark cycle.

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Experimental design. During daytime hours, roosters were found to be infected with a mesostigmatid mite that had legs in the anterior portion of its body. This morphology, along with consultation with an entomologist, established a diagnosis of NFM. Throughout the duration of the experiment, roosters were housed in 2 separate pens based on experimental condition; these pens were roughly 20 ft apart in a previously depopulated and disinfected isolation wing at the Poultry Research Laboratory at the University of Wisconsin-Madison. Measures to prevent cross-contamination between these pens included spatial distance between pens and changing personal protective equipment (lab coats, gloves, and shoe covers) when moving from one pen to the other. Three experiments were performed by using 2 topical spray treatments—a commercially available formulation of *Beauveria bassiana* spores suspended in mineral oil (balEnce, Terregena, Raleigh, NC) and a tetrachlorvinphos–dichlorvos product (RAVAP-EC, KMG Chemicals, Houston, TX)— with water as a control.

Treatments were applied according to the manufacturer's label directions. The RAVAP-EC label limits treatment dosage to 30 mL (1 oz) per bird and states that the treatment should not be repeated more often than once every 14 d. Therefore, during the first 2 experiments, approximately 30 mL of each agent (water, RAVAP-EC, or *Beauveria*) was used per bird and was applied twice, with a 14-d interval between treatments. This regimen resulted in a per-treatment dose, by using label dilutions, of 137 mg tetrachlorvinphos and 31.5 mg dichlorvos per bird for RAVAP treatment and 2.9×10^{10} spores per bird for *Beauveria* treatment. Because the *Beauveria* manufacturer suggested a target of 2.5×10^8 spores per bird, the 30-mL volume was considered to be appropriate. During each treatment, birds were restrained by their hindlimbs while the keel was supported by using a plastic slotted crate; the test material then was applied topically by pump pressure spray bottle. Separate bottles were used to apply each treatment, with bottle demarcations being used to determine the quantity applied to each bird. Treated areas included the vent, back, back of the head, and under the wings, with care to ensure penetration beneath the feathers to the level of the skin but avoiding contact with the eyes. Treatments were applied on days 0 and 14 for the first 2 experiments, and on days 0, 7, and 14 for the third experiment, which included only the fungal treatment. For each experiment, observations were taken over a period of 8 wk, during which each bird was scored every 7 d (and prior to any scheduled treatment) according to a standardized visual index (Figure 1).

In the first experiment, the roosters were divided randomly into 2 groups ($n = 7$ per group), one of which received water, whereas the other received *Beauveria*. Once the 8-wk observation period had ended and mite levels returned to pretreatment levels, the birds were sorted randomly into 2 new groups and allowed to acclimate to one another prior to beginning the second experiment. In the second experiment, which began 7 d after the first ended, one group received *Beauveria* and the other received RAVAP-EC.

After completion of the second experiment's observation period, a third experiment assessed the efficacy of a greater dose (roughly 300 mL [10 oz] or 2.9×10^{11} spores per bird) and increased frequency of application (3 applications 7 d apart at days 0, 7, and 14) of *Beauveria.* This experiment excluded the 7 birds that had been exposed to RAVAP-EC and began once the mite levels of the remaining 7 birds had returned to their original pretreatment levels (day 56 of experiment 2 was day 0 for experiment 3).

Scoring index. The scoring index was patterned after a previously published scheme,[31] which was validated by using feather digestion (Figure 1). The scale ranges from 0 to 3, with increments of 0.5 allowed for intermediate scores. A score of 0.5 would indicate as few as 1 or 2 live mites, with scores of 1, 2, and 3 being assigned to mild, moderate, and severe levels of infestation, respectively. The head, neck, and back (defined as the 'head' score) were scored as a single 'region,' whereas the vent (including area just dorsal to the tailfeathers but caudal of the uropygial gland) was scored as a separate region. The researcher scoring the mite levels each week was blind to the treatment group and previous scores of each bird for the first 2 experiments and to the previous scores of each bird for the third. Although a single researcher was the principal scorer of mite level at any data collection point, another researcher was trained on the scoring system in the event that the principle scorer could not be present, which occurred twice during the entire study. During that training, the scores between these 2 researchers were analyzed and found to not be significantly different.

Statistical analysis. All statistical analyses were conducted by using the PASW Statistics package version 17.0 (formerly SPSS Statistics, Chicago, IL). For all 3 experiments, data were analyzed by using repeated-measures ANOVA, with time as a within-subjects factor. A sample size of 14 birds was determined based on a power analysis by using G*Power software (http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/). Input parameters assumed power of 0.95, a large effect size (0.8), 8 planned measurement points, and 2 study groups. Group comparisons were conducted in experiments 1 and 2, with treatment type as a between-subjects factor. Statistical significance was set at a P value of less than 0.05.

Results

Experiment 1: *Beauveria* compared with water. Figure 2 depicts ratings of NFM infestation of roosters across a period of 8 wk comparing the control treatment (water) against *Beauveria* (30 mL per bird, applied on days 0 and 14). Analyses were conducted separately for the head and vent regions. For the head region, results revealed a significant main effect of group, with the *Beauveria* treatment resulting in significantly lower mite scores ($F[1,12] = 6.07$, $P < 0.05$). In addition, a significant time × group interaction ($F[7,84] = 5.41$, $P < 0.01$) was present such that the *Beauveria* group demonstrated lower mite levels than did the control group as a function of time. Results for the vent region were nearly identical, with a significant main effect of group ($F[1,12] = 12.35$, $P < 0.01$) and a significant time × group interaction ($F[7,84] = 11.70$, $P < 0.001$).

Two applications of *Beauveria* at 30 mL per bird did not completely eliminate the mite infestation, which returned to pretreatment levels by the end of the observation period (8 wk). For both the head and vent regions, the time × group interaction showed significant ($P < 0.001$) quadratic trend, indicating that mite levels were similar across the 2 groups at the outset and end of the observation period but were markedly lower in the *Beauveria* group during the weeks immediately after the treatment.

Experiment 2: *Beauveria* compared with RAVAP-EC. Experiment 2 investigated the effect of equivalent doses of *Beauveria* and RAVAP-EC (30 mL per bird, applied on days 0 and 14) over a period of 8 wk (Figure 3). The group of roosters treated with RAVAP-EC was clear of mites by the second week and did not have visible live mites by the end of the observation period. The *Beauveria*-treated group experienced a reduction in mite

Score	Mite load	Other criteria
0	No live mites observed	Evidence of previous mites may be present, but no live mites are detected after agitation of feathers
1	Mild; few live mites observed, usually affecting only 1 or 2 sites per region	Scant evidence of mites (frass, excrement, clumping of feathers)
2	Moderate; intermediate numbers of live mites observed over several sites within a region	Evidence of mites (frass, clumping) and scaling present
3	Severe; numerous live mites observed over the majority of a region	Evidence of mites, scaling, and scabbing present

Figure 1. Scoring system used to evaluate mite infestation level.

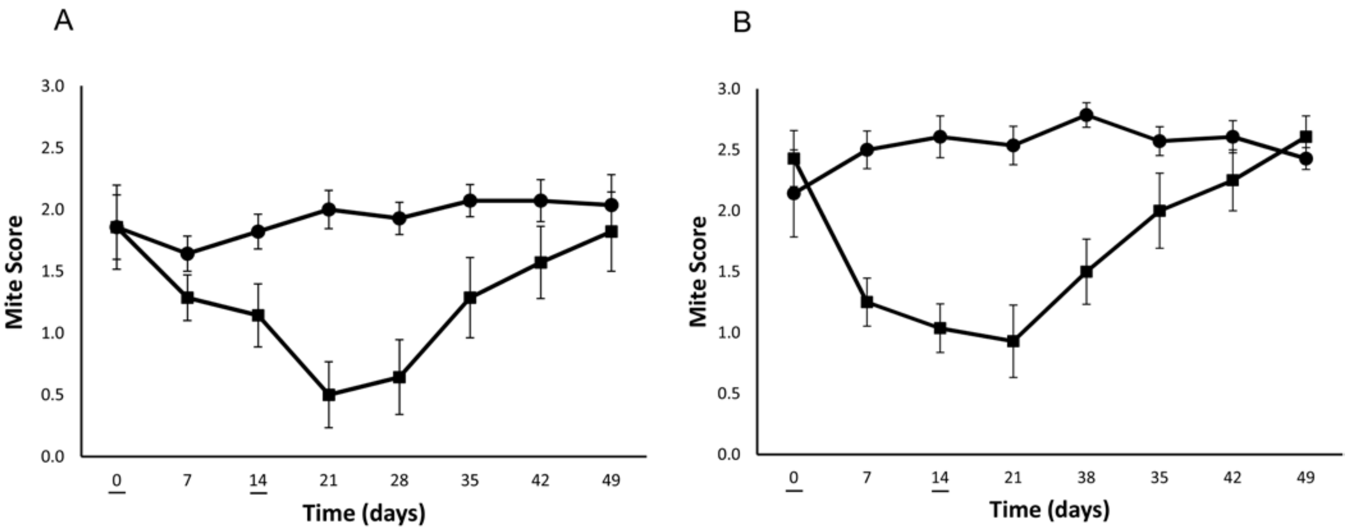

Figure 2. Mean mite infestation scores by treatment region for roosters treated with 30 mL (1 oz) *Beauveria bassiana* (squares) compared with a water control (circles) over an 8-wk observation period. (A) Head region. (B) Vent region. Underlined weeks indicate treatment dates. Error bars indicate SE.

levels, which returned to pretreatment levels by the end of the observation period. For the head region, statistical analyses revealed a significant main effect of group ($F[1,12] = 26.09$, $P < 0.001$) and time × group interaction ($F[7,84] = 4.29$, $P < 0.05$), with lower mite scores as a function of time in the RAVAP-EC group. For the vent region, the main effect of group ($F[1,12] = 323.17$, $P < 0.001$) and group × time interaction ($F[7,84] = 30.14$, $P < 0.001$) again were significant.

Experiment 3: Increased dosage of *Beauveria*. The final experiment explored the effect of a higher dosage and application frequency of *Beauveria* on mite scores across an 8-wk observation period (Figure 4). With a weekly dosage of 300 mL per bird applied on days 0, 7, and 14, mite levels were reduced to a point at which visual inspection on day 21 failed to detect any live mites on any treated bird. Mite levels began to increase during weeks 4 through 7 after ceasing the weekly treatment. Repeated-measures ANOVA for the head region indicated a significant effect of time ($F[7,42] = 24.29$, $P < 0.001$), with significant ($P < 0.001$) quadratic trend for this effect, indicating lowering of mite levels in response to treatment and subsequent increase after completing treatment. The vent regions similarly showed a significant time effect ($F[7,42] = 29.13$, $P < 0.001$), with significant ($P < 0.001$) quadratic trend.

The data from the third experiment indicate that increasing the dose and frequency of *Beauveria* application significantly increases its efficacy with respect to mite level. Although mite elimination was not fully sustained after ceasing treatment, mite levels on day 49 (35 d after the last treatment) remained lower than those for day 0 for both head ($t[6] = 4.60$, $P < 0.01$) and vent ($t[6] = 5.79$, $P < 0.01$) regions.

Discussion

The aim of the present study was to evaluate the efficacy of a commercial preparation of the entomopathogenic fungus *Beauveria bassiana* against NFM in poultry. Although *Beauveria* proved more effective than a water control, at equivalent volumes the RAVAP-EC treatment was most effective at lowering mite levels. However, increasing the dose and frequency of application of *Beauveria* increased its efficacy such that visual inspection failed to detect any mites on the bird. Although mites subsequently reinfected the *Beauveria*-treated group, this reinfection most likely was secondary to continued environmental pressure, such as live mites in the bedding or persistence of mite eggs on feather shafts. This situation highlights the need for environmental treatment and enhanced individual bird therapy if *Beauveria* is to be useful in controlling mite populations on poultry.

The NFM is a longstanding concern in the poultry industry; a pair of Italian scientists first characterized this mite in 1877.[7] The mite was first recorded in North America in Beltsville, MD, in 1917, moving to Raymond, IL, and the rest of the United States by 1919.[30] The longstanding nature of the challenge that the NFM poses is explained in part by its biology. After taking a blood meal, female NFM lay clutches of as many as 5 eggs along a feather shaft, which then hatch into larvae and progress to protonymphs and deutonymphs, reaching adulthood in as few as 5 to 7 d.[20] In addition, NFM spread in a variety of ways,

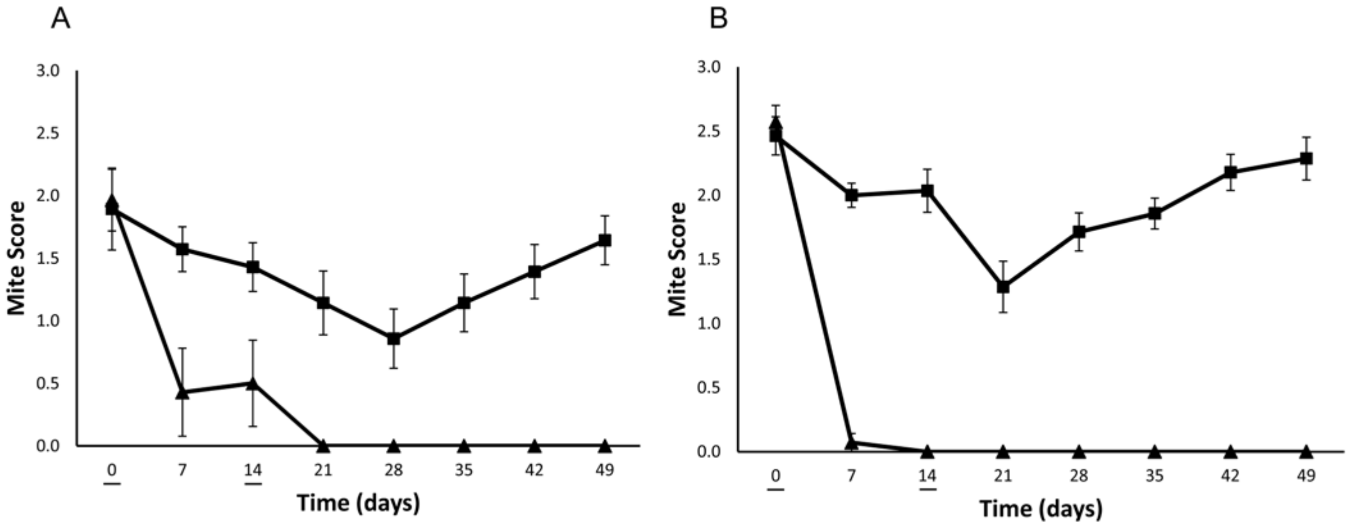

Figure 3. Mean mite infestation scores by treatment region for roosters treated with 30 mL (1 oz) *Beauveria bassiana* (squares) compared with 30 mL (1 oz) RAVAP-EC (triangles) over an 8-wk observation period. (A) Head region. (B) Vent region. Underlined weeks indicate treatment dates. Error bars indicate SE.

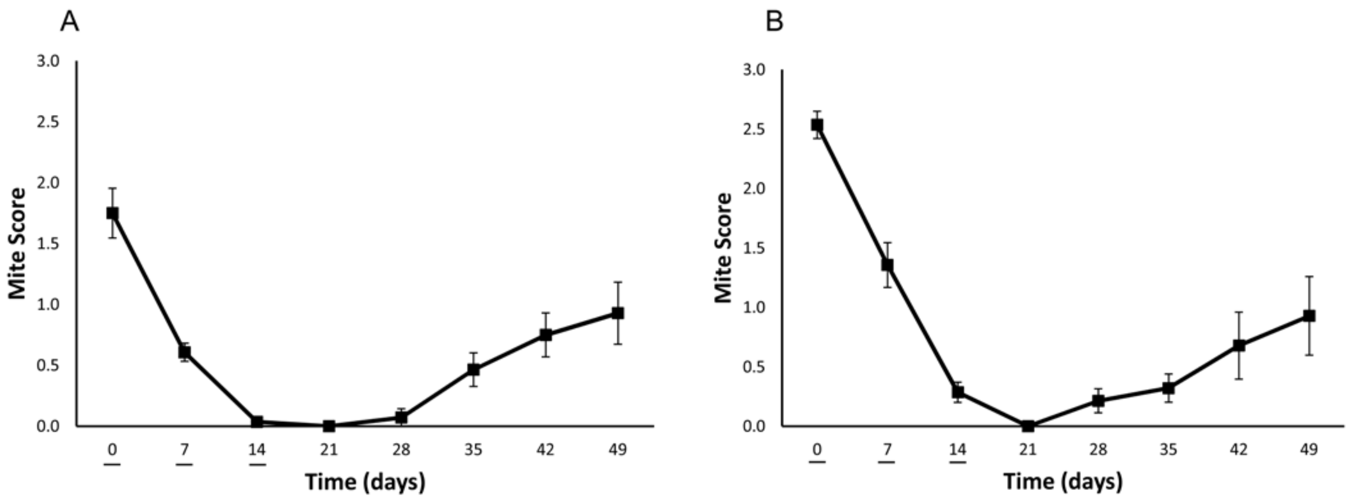

Figure 4. Mean mite infestation scores by treatment region for roosters treated with 300 mL (10 oz) *Beauveria bassiana* over an 8-wk observation period. (A) Head region. (B) Vent region. Underlined weeks indicate treatment dates. Error bars indicate SE.

from contaminated equipment[2] or environments[18] to alternate hosts such as wild birds[20] and rodents.[16,23] Even pullet-rearing facilities have been implicated as a potential source of mites spread to previously unaffected animals in distal facilities.[19]

Although interstrain and even interindividual variations in susceptibility have been reported,[6] roosters generally are more susceptible to infestation than are hens.[2,20] This sex-associated predilection may provide some benefit to NFM, because in a pen setting or the wild, roosters will mate with many hens and thus aid in distributing the mite. In addition, NFM tend to congregate in specific locations on the birds, clustering at the base of feathers in the vent and occipital regions.[2,20] This localization is likely a reflection of the difficulty a bird has in grooming these areas; other investigators have found a positive correlation between mite numbers and beak trimming, suggesting that intact beaks enhanced the birds' grooming ability and thereby reduced the mite load.[24]

The NFM poses multiple concerns to investigators using poultry models for agricultural or biomedical research. Previously, research on the effect of mites in commercial poultry production had been mixed, with some authors finding decreases in egg production and feed consumption[1,2] and others disagreeing.[10] However, a recent report documented significant costs to poultry users, including decreased egg production, egg weight, and feed conversion efficacy, as the immune system upregulates to address mite infestation.[26] More direct effects to chickens include anemia, decreased body condition, and, in cases of severe infestation, even death.[20]

An additional concern is the hazard of NFM to poultry professionals, including animal research technicians. *The Guide for the Care and Use of Agricultural Animals in Research and Teaching* insists that the welfare of the animal caretaker should be considered in evaluating the environment of poultry.[12] Animal care workers are at increased risk for allergic respiratory disease[21] and dermatitis secondary to bites from indiscriminant mites, as well as general discomfort associated with the crawling of mites across the caretakers' skin.[20]

Due to concerns such as these, prevention is a key part of an integrated pest management program for poultry in the research setting. With respect to NFM, preventative measures such as blocking rodents and wild birds from accessing the poultry facility and ensuring sanitation of fomites such as egg crates are recommended.[2] However, when these measures fail, veterinarians and facilities frequently turn to chemical treatments for control. Commonly used pesticides include permethrin, a pyrethroid that blocks depolarization of axonal sodium channels; tetrachlorvinphos–dichlorvos combinations (RAVAP-EC) and malathion, which are organophosphates that inhibit the activity of cholinesterase at the synapse; and carbaryl, a carbamate that inhibits cholinesterase.[27] As a final measure, depopulation followed by resting the facility for several months can break a mite infestation, provided that the fallow period outlasts the mites, which can live off the host for as long as 2 to 3 wk.[15]

Despite the availability of chemical control, multiple challenges exist in treating NFM in poultry. Some of the aforementioned products are slowly being pulled from the market because of environmental concerns (carbaryl) or are losing their efficacy as mites become resistant (permethrin).[27,31] Organophosphates pose considerable concern to both birds and personnel, who risk experiencing not only acute toxicity if accidentally overexposed but also neurobehavioral changes when exposed at chronic, subclinical levels.[13] In addition, because pesticides are regulated by the US Environmental Protection Agency and not the US Department of Agriculture, veterinarians do not have the ability to deviate from the label restrictions by using tools such as the Animal Medicinal Drug User Clarification Act. In the case of the organophosphate RAVAP-EC, this restriction means that any application must occur at no less than a 2-wk interval. Restrictions such as these may hinder effective treatment, given that the time between the hatching of mite eggs (which are resistant to the effects of chemical pesticides) and adulthood is half that duration.

Researchers using poultry face particular challenges with regard to chemical treatment of mite infestations because these products may confound results, as a researcher at our facility directly observed during a developmental biology project. Moreover, when protecting multiple genetic lines or confronting a lack of facility space, researchers and facility managers may be unable or unwilling to depopulate and repopulate to combat NFM infestation. These concerns, in addition to the growing market for organic foods, have helped drive the exploration of novel treatments, including garlic oil[5,31] and limonene.[8]

One such novel treatment is the entomopathogenic fungus *Beauveria bassiana*. *Beauveria* is effective against *Rhipicephalus microplus* (cattle ticks),[28] *Musca domestica* (house flies),[17] *Alphitobius diaperinus* (lesser mealworms),[9] and *Dermestes maculatus* (hide beetles).[14] In the case of cattle ticks, *Beauveria* isolates decreased the number of eggs laid as well as the percentage of larval hatching and were shown to be compatible with the pesticide amitraz.[28] Isolates of *Beauveria*, which can be found in soil, are selected for their ability to colonize the insect or arthropod in question and then are propagated. This selection may underlie other findings indicating that the fungus does not appear to harm certain beneficial insects.[17] *B. bassiana*'s mechanism of action involves the production of organic acids (such as oxalic and citric acids) and hydrolytic enzymes (proteases and chitinases), which help solubilize the cuticle of insect and arthropodan hosts, making them more susceptible to penetration by fungal hyphae and subsequent fatal digestion of the pest.[3,4,11] In addition, *Beauveria* appears to be nonhazardous to humans,[29] although the possibility for developing allergies does exist.[22]

In the present study, experiment 1 demonstrated that *Beauveria* resulted in significant reduction of mite levels. However, at a dosage of 30 mL (2.9×10^{10} spores) per bird, treatment with *Beauveria* did not fully eradicate mites from any rooster nor was its effect sustained throughout the observation period. The average mite score did not drop below 1 in the treatment group, and mite levels had returned to original, pretreatment levels by the end of 8 wk (Figure 2).

In the second experiment, the same roosters were resorted randomly into 2 new groups after mite levels had returned to pretreatment levels. One group was treated with the organophosphate RAVAP-EC, whereas the other was treated with *Beauveria*. The organophosphate eliminated live mites by the third week of treatment, and mites were not detected on any bird for the remainder of the 8-wk observation period. The *Beauveria* treatment again reduced, but did not eliminate, observable mites, and mite scores had returned to original, pretreatment levels by the end of the observation period (Figure 3).

Experiment 2 prompted questions regarding the dose and frequency of *Beauveria* treatment. The amount applied and the frequency of application in the first 2 experiments both were dictated by the organophosphate label, in an effort to minimize variability for the purposes of comparison. However, due to its relatively nonhazardous nature,[29] the fungal treatment offers the ability to increase the amount applied to each bird and, more importantly, shorten the interval between reapplication to catch mites as they hatch from eggs. Experiment 3 demonstrated that, when the amount applied per bird was increased to 300 mL and the reapplication period was shortened to 7 d, reduction in mite levels was more comparable to those obtained by using the organophosphate, with visual inspection failing to detect mites on the birds within 21 d of starting treatment (Figure 4). Although the effects observed in experiment 3 could be attributable to repeated exposure of the mite to the fungus over the course of the 3 experiments, the fact that a reduction in mites was observed in all birds in the third experiment group, followed by an increase in mite levels when treatment was discontinued, suggests that the increased dose and frequency are responsible for the results.

The underlying reason for the return of NFM to the *Beauveria*-treated group but not the organophosphate-treated group is open for discussion. Perhaps the organophosphate has greater residual effects than does the fungus; using mineral oil as a dispersant to increase the time the fungal spores stay on the bird and therefore in contact with mites may increase the efficacy of *Beauveria* treatment. Possible undesirable secondary effects, such as coating birds with litter, precluded exploration of this technique in the present study. Other potential reasons for the return of NFM to *Beauveria*-treated roosters include failure of the fungus to target mite eggs and insufficient sensitivity of the observation system to detect NFM persistence past treatment.

The present findings underscore the need for additional research to better characterize the effectiveness of the *Beauveria* fungus as a potential treatment for mites in agricultural and research settings. In addition to improving therapy directed at birds, examining the environmental aspects of infection (such as including the bedding and roosts in spraying, or simply removing birds to a clean facility once they are visually negative for mites) likely would be particularly beneficial, because doing so may be important from the perspective of sources of reinfestation. In addition, alternative media (such as mineral oil instead of water) to deliver the spores should be explored. This adaptation may improve the duration of effect, because mineral oil likely would cling to birds longer than would water, thereby maintaining an elevated level of spores on the animals; however such use may be limited to caged birds not maintained on litter.

An important limitation of the present study was the relatively small sample size; additional work is needed with larger numbers of birds to confirm the findings here. Furthermore, direct comparison of the organophosphate treatment at label directions with *Beauveria* treatment at the more effective dose and frequency would be valuable. In addition, although topical application of *Beauveria* spores is unlikely to affect study variables or prompt concerns regarding residues, these assumptions should be verified, and what effect, if any, *Beauveria* has on the biology of chickens themselves should be confirmed. Finally, the interaction between this entomopathogenic organism and other pesticides needs to be explored. *Beauveria* has been shown to be synergistic with other pesticides, including amitraz,[28] and *Beauveria* treatment could be useful in preventing the development of resistance if used in a rotational manner with other effective therapies, where possible.

Although additional research is needed to further characterize the use of *Beauveria* as an effective control of NFM in poultry, the results presented here suggest that this fungus is effective at reducing mite levels and (at sufficient dosages) could become an important part of an integrated pest management program. The ability to control or eliminate NFM from poultry in research facilities by using a method that preserves valuable research data, minimizes potential harm to both birds and personnel, and has minimal negative environmental impact would prove to be invaluable.

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