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Authors: Smits, Judit E., Johnson, Dan L., and Lomer, Chris

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PATHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF RING-NECKED PHEASANT CHICKS FOLLOWING DIETARY EXPOSURE TO THE FUNGUS *METARHIZIUM FLAVOVIRIDE,* A BIOCONTROL AGENT FOR LOCUSTS IN AFRICA

Judit E. Smits[1], Dan L. Johnson[2], and Chris Lomer[3]

[1] Department of Veterinary Pathology, Western College of Veterinary Medicine, and Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada S7N 5B3 (Smits@skyway.usask.ca);
[2] Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada T1J 4B1 (JohnsonDL@em.agr.ca);
[3] Center for Biological Control in Africa, International Institute of Tropical Agriculture, BP 08 0932, Cotonou, Benin (C.lomer-T@cgnet.com)

ABSTRACT: *Metarhizium flavoviride,* a fungal pathogen of grasshoppers and locusts, appears to be an effective, non-chemical insecticide (mycoinsecticide) for control of grasshoppers and locusts. This study, conducted during June and July, 1997, examined the pathogenic potential of this entomopathogenic fungus to non-target avian species that encounter infected insect prey items or contaminated food sources. Ring-necked pheasant (*Phasianus colchicus*) chicks were exposed to one of three diets, (spore-coated feed, infected insects, or untreated feed), either from 4 to 9 days of age, or, from 35 to 40 days of age. Necropsies were conducted on birds 10 days and 46 days old, respectively. Neither consumption of infected insects, nor of spore-coated feed, resulted in pathological changes, or significant changes in weight, growth rate, behavior, or mortality rate. Histological examination of organs indicated either no changes related to treatment, or normal tissue responses to antigenic challenge.

Key words: Entomopathogen, grasshopper, locusts, *Metarhizium flavoviride*, mycoinsecticide, pathology, *Phasianus colchicus,* ring-necked pheasant.

INTRODUCTION

Environmental and health concerns regarding the methods used to combat large-scale insect pest infestations have resulted in renewed interest in development of effective control agents with reduced toxicity to non-target organisms. Grasshoppers and locusts are often the most serious agricultural pests in grassland biomes of the world, such as Sahelian Africa (Geddes, 1990), and the plains of North and South America. Between 1985 and 1991, grasshopper infestations necessitated spraying of 7.7 million ha in Saskatchewan and Alberta, with an economic loss of at least $326 million (Johnson et al., 1996). In the USA, grasshopper infestations on rangeland have been estimated to reduce grazing potential by $400 million in 1 yr (Hewitt and Onsager, 1982). The most recent outbreak of desert locust (*Schistocerca gregaria*) and related acridids in Africa beginning in 1988 affected 23 nations, and required over $250 million in donor aid for pest control and relief for damage caused by locusts (Showler and Potter, 1991; Showler, 1995).

Significant impacts of chemical insecticides on avian wildlife have been reported as a result of control actions during outbreaks. Recent grasshopper infestations of pastures and hayfields in Argentina are reported to have resulted in pesticide poisoning of at least 4,000 Swainson's hawks (*Buteo swainsoni*) during only a few weeks of spraying; regional losses for this period are estimated at 20,000 hawks (Zaccagnini et al., 1998). Grasshopper insecticides have been implicated in the deaths and/or disappearance of endangered burrowing owls (*Athene cunicularia*) (James and Fox, 1987), resulting in the deregistration of carbofuran for grasshopper control in Canada (AAFC, 1993; PMRA, 1995).

As part of a world-wide search for alternatives to chemical insecticides used to control these pests, isolates of the fungus *Metarhizium flavoviride* have been investigated for control of grasshoppers and lo-

custs (Lomer et al., 1997). It is highly virulent to grasshoppers and locusts, and has been chosen for development by the LUBILOSA Project ("Lutte Biologique Contre les Locustes et Sauteriaux") for use in Africa (Prior et al., 1992). Spores are produced on rice, separated, dried and vacuumed sealed. They can be stored for 1 yr at warm ambient temperatures of 27 to 32 C, or for longer periods at lower temperatures. Spores are applied in oil spray droplets. Recent field experiments of *M. flavoviride* application have achieved about 70% mortality of grasshoppers 10 to 12 days after treatment (Lomer et al., 1997). This level of control of grasshoppers has been identified in other studies as an ideal target range for efficacy, because it leaves a food resource sufficient for survival and reproduction of grassland songbirds (Johnson et al., 1996) while limiting significant economic damage by grasshoppers. Because the remaining insects are preyed upon by vertebrate predators such as birds, data on nontarget safety of the dried conidia of IMI 330189 (International Mycological Institute, Surrey, UK), formulated and applied as an ultra low volume (ULV) oil suspension, is required for large-scale field testing and registration.

Metarhizium flavoviride is more active at higher temperatures than other entomopathogenic fungi such as *Beauveria bassiana,* allowing *M. flavoviride* to be more effective in hot, sunny conditions that stimulate basking by the target insects, and subsequent elevation in their body temperature in excess of 35 C. This fungus might be expected to be nonviable at vertebrate body temperatures. In a challenge study in which *B. bassiana* fungal spores were injected into skeletal muscles of mice, the closely observed progression of pathological response and repair at the injection sites showed a pattern consistent with an inflammatory response to foreign particulate matter (Semalulu et al., 1992). The reaction showed no evidence of active infection by the fungus. As early as three days post inoculation, there were no viable spores recoverable from injection sites. The activity of *M. flavoviride* at higher temperatures is an asset for control of grasshoppers and locusts in warm seasons, or warmer regions of the world. However, because *Metarhizium* spp. are viable within the range of mammalian and avian body temperatures, investigation of possible impacts on vertebrates becomes important, similar to the toxicity testing required before chemical insecticides can be considered for locust control (Ritchie and Dobson, 1995).

Published information is lacking on the effects of *M. flavoviride* on vertebrate species such as birds or reptiles which are at risk because they inhabit the same grassland ecosystems as do acridids. The purpose of this study was to examine the pathogenic potential of the entomopathogenic fungus *M. flavoviride* to non-target avian species, using environmentally relevant routes of exposure. Two realistic routes of exposure for birds would be through feed contaminated with spores from *M. flavoviride,* or through prey items such as grasshoppers, which had died from infection by *M. flavoviride.*

MATERIALS AND METHODS

Fungal isolate

Metarhizium flavoviride has been collected from numerous insects in the humid and Sahelian zones of Africa. Isolate IMI 330189, was developed by LUBILOSA. This isolate is indigenous to West Africa, where it was originally isolated from the grasshopper *Ornithacris cavroisi* collected near Niamey, Niger in 1988. It was subsequently tested at the International Institute of Biological Control (Ascot, UK), the International Institute of Tropical Agriculture (Cotonou, Benin) and CILSS Département de Formation en Protection de Végétaux (Niamey, Niger) (Lomer et al., 1997). Spores were obtained from the LUBILOSA formulation facility (International Institute of Tropical Agriculture, Cotonou, Benin).

Avian test species and treatments

For this study, conducted during June and July 1997, uniform-sized, 2-day-old ring-necked pheasant chicks (*Phasianus colchicus*) were obtained from mass-reared broods at the

Brooks Wildlife Center (Alberta Forestry, Lands and Wildlife, Brooks, Alberta, Canada), using the same stock and methods as birds used in previous assessments of avian toxicity of grasshopper insecticides (Martin et al., 1995). Chicks were confined in the Controlled Environment Barn of the Lethbridge Research Centre (Lethbridge, Alberta, Canada), on a 15 hr light; 9 hr dark cycle, in groups of 10, in new wooden pens (113 cm × 50 cm × 60 cm high). A total of 18 pens were used for various facets of the experiments, 12 of which were reserved for treatment of the birds destined for histopathology analysis. Pens were open at the top, and equipped with two 100-W brooder lamps, water, and feed trays. Light height was adjusted to provide a floor level air temperature of about 32 C. Ambient room temperature outside the cages was 25 to 26 C. Pen floors were covered with plastic, and overlain with 5 cm of woodchip bedding, which was removed and replaced each week. Pheasant chicks were fed unmedicated duckling starter (Unifeed, Lethbridge), 21% protein; 3% crude fat; 5% crude fibre; supplemented with vitamins A, E, D. Duckling starter was replaced with a 16% protein poultry finisher ration (Unifeed) after 3 to 4 wk. Unmedicated water was available *ad libitum.*

Pheasants were randomly assigned to one of three treatments (ingestion of spore-coated feed, ingestion of infected insects, or untreated control insects) and labeled with color coded leg bands to ensure that clinical assessments, necropsies, measurements and histopathology observations were conducted without bias. Each of the four complete replications included 30 birds, with 10 birds, in most cases five of each sex, per treatment (one pen = one experimental unit). Treatments were assigned randomly to pens within each replication (by order, 123, 213, 132, 231, 213, 321). The experiment was replicated four times, with a total of 120 birds available for the histopathology portion. Measurements on individual birds included initial and final body weight, tarsus length, and mortalities. Chicks were observed daily for clinical signs of physiological or pathological effects associated with treatment, including unusual vocalization or blinking, change in gait or coordination, other neurological signs, respiratory distress, anorexia, postural changes, ruffled or denuding of feathers, prolonged sternal recumbency, weakness, or decreased responsiveness to investigator presence. Observers also watched for and recorded any evidence of gastrointestinal disorder, nasal or ocular discharge, cyanosis of skin or mucous membranes, skin lesions, or emaciation. Upon completion of the dietary exposure, sterile cotton tipped swabs (3 per bird) were used to wipe the beaks, feet and feathers of four randomly selected birds from each treatment group. These swabs, as well as samples of feces and blood were tested for the presence of *M. flavoviride,* by plating smears on a selective medium containing agar, bile, glucose, peptone, rose bengal, Dodine, chloramphenicol, cyclohexamide (Veen and Ferron, 1966). Fresh stained and unstained samples of feces and blood were examined using Nomarski differential interference contrast microscopy.

Insect food items

Fourth-instar nymphs of the migratory grasshopper, *Melanoplus sanguinipes,* were determined in preliminary tests to be readily accepted prey items of ring-necked pheasants (D. L. Johnson and J. E. Smits, unpubl. data). The insects were hatched from eggs laid by field-collected adult grasshoppers, and reared on a diet of wheat leaves, lettuce and wheat bran. The insects used as food in the treatment group were infected prior to feeding to birds, using methods that simulate application of spores in operational situations, that result in optimum contact and efficacy (Bateman et al., 1993). Dry spores of *M. flavoviride* IMI 330189 were formulated in sunflower oil as detailed in Inglis et al. (1997) and applied to 500 15-day-old grasshoppers and their wheat-leaf food in glass cages, using a hand-held mist sprayer calibrated before and after application. Spores were formulated based on the dry spore density per unit weight determined by the production facility, adjusted by viability as indicated by germination tests at the time of formulation. Dispersion and concentration of conidia in the formulated oil were determined with a hemocytometer and microscopic examination. Droplet capture on oil-sensitive paper and glass slides was utilized to estimate that the viable spore application load was approximately 5×10^4 to 1.2×10^5 viable spores per insect, plus smaller quantities contacted during feeding on the day of treatment. Within 5 to 7 days, all the infected grasshoppers typically exhibited symptoms of successful infection by *M. flavoviride,* ranging from slight lethargy to discoloration and death. Mortality of the treated grasshoppers was greater than 90% by day 9 post-inoculation. One hundred percent of the unused, treated insects died, whereas less than 5% of the untreated insects from the control groups died. The grasshoppers, chosen at the stage at which they were judged to pose the maximum hazard to predators (i.e., moribund or recently dead), were fed to the pheasants 7 to 12 days after infection. During the treatment period, birds were individually confined in transparent,

3.5 L cylindrical cages with two to three infected grasshoppers for 5 to 15 min, to ensure that each bird consumed the allotted amount. Each bird consumed a total of 12 infected grasshoppers weighing about 0.24 g each, over the treatment period of 5 days.

The spore-treated feed (simulating an hypothesized alternative route of exposure of the birds to *M. flavoviride*) was prepared by mixing and tumbling dry spores with dry feed at the rate of 10 g of dry spores per 500 g of duckling starter. Control and "infected insect" treated groups received only clean duckling starter supplement, as did the "spore-treated feed" group, except during the 6 days of treatment. Feed was provided in all the pens *ad libitum*, in trays that each held 130 g.

Histopathology

The impacts of the treatments of spores in food and infected insects as food were assessed in pheasants at two ages. In the first test, birds were fed challenge diets between 4 and 9 days of age, euthanized and necropsied at 10 days of age. In the second test, birds received the challenge diets between 35 and 40 days of age, and were necropsied at 46-day-old.

In the 9-day-old group, one male and one female from each treatment group were randomly selected for pathological examination. One additional, grasshopper-exposed, bird which had died unexpectedly on the day of the planned sampling was also examined histologically, making a total of 25 9-day-old birds (2 birds × 3 treatments × 4 replications + 1). In the 46-day-old group, one male and two females from each treatment group were randomly selected for pathological examination (n = 35 birds, 3 birds × 3 treatments × 4 replications − 1 not used). The pheasants were euthanized in a pure atmosphere of CO_2 followed by cervical dislocation. Tissue samples were taken of liver, thymus, bursa of Fabricius, spleen, lung, crop, proventriculus, and cecum. Samples were immediately fixed in 10% neutral buffered formalin, subsequently embedded in paraffin blocks, sectioned (6 μm), mounted on glass slides, stained with haematoxylin and eosin or Gomori's methenamine silver nitrate stain, and examined for histopathology that could have resulted from mycotic infections or mytotoxin damage (Scott et al., 1985). Deviations from normal, healthy tissue, such as inflammation, necrosis, alterations to normal cellular structure, disruption of tissue architecture, or evidence of invasion by infectious agents, were noted if present. Tissue samples were scored using a numbering system of 1 to 4. Tissues with no abnormal findings, or mild artifactual tissue changes, were rated as "1". Tissues showing areas with mild pathological changes, or up to two small foci of more distinct pathology were rated "2". A score of "3" indicated that there were numerous mild, or at least one large lesion, and any tissue with severe or extensive pathological change was rated "4". Identification of the individuals' tissues was coded so that during necropsy examination and histopathology, the pathologist was unaware of the treatment received by any of the birds.

Statistical analyses were done using SAS (1990) software.

RESULTS

Clinical assessments and histopathology

All the birds readily consumed the spore-treated feed or infected insects during the period of exposure to the experimental diets. The presence of colony-forming units of *Metarhizium* was detectable from wipes taken from birds' beaks, feet and feathers, but none of the fecal and blood smears on the selective media showed evidence of *Metarhizium* colonies (40 plates). Only small numbers of colonies of *Penicillium* sp., *Rhizopus sp.*, *Mucor sp.*, and bacteria were observed on the plates.

With the exception of two cases of pecking of smaller birds, no cases of unusual clinical or behavioral comportment were noted, either in the undisturbed birds, or in response to the presence and activities of the observers. There was minor initial mortality in all replications and treatments, but this was not significantly different among experimental treatment groups. Death losses in the assigned groups used as the sources of samples for the histopathology analysis totalled 2/40 in the control group, 3/40 in the infected insect group and 1/40 in the infected feed group; no mortalities were observed during the treatment period or between treatment and euthanasia for necropsy examination.

Overall, there were fewer changes in the tissues of the pheasants exposed prior to ten days of age, compared with those exposed around 40 days of age. In the younger birds, only three of 25 birds had any tissue changes which ranked above a mod-

erate deviation from normal, and these changes generally represented an increase in inflammatory cell populations, rather than serious pathology. Among the older birds, 16 of 35 birds had at least one tissue with a rank of "3" (indicating numerous mild lesions, or the infrequent presence of moderate lesions) or greater, but of these, five were from the control group. Histopathology of the samples from each group revealed no differences in any of the experimental groups. Among the younger group of 25 pheasants, two of nine birds exposed to grasshoppers infected with *M. flavoviride,* and three of seven birds exposed to spore-coated feed had some germinal centre development (one to five germinal centres) in splenic tissue, while none had formed in spleens of any of the control group birds.

In the older group of pheasants, higher spleen weights seen in the spore-coated feed group (see below) made it relevant to describe histological findings in the spleens, even though they were not statistically different among treatments. The only histologically detectable difference in the feed-exposed birds, compared with those on the other two diets, was the very low splenic germinal centre formation evident in half the birds in this group (six of 12), compared with only two of 12 in each of the infected insect and control diet groups. Apart from this, the major features accounting for the variable spleen scores, and possibly spleen weights among treatment groups were the overall splenic cellularity, and the relative amounts of white and red pulp which make up the bulk of the parenchyma. Overall in both groups of birds, there were pathological changes in several organs, but these were not consistently associated with a particular treatment group.

Body and organ weight

Body weight differed weakly between the sexes in the early treatment group. Mean body weights of birds 10- to 11-day-old was 90.4 g (SD = 13.1, n = 48) for male pheasants and 82.7 g (SD = 12.3, n = 49) for females (ANOVA, $F_{1,3} = 7.76$, $P = 0.069$. Differences due to sex were tested using block X sex interaction as the F denominator, for this and for the following comparisons of variables in this randomized complete block design). Male tarsal length (4.11 cm, SD = 0.228) was slightly greater (ANOVA, $F_{1,3} = 6.97$, P = 0.078) than for the females (3.99 cm, SD = 0.236).

Males necropsied at 46 days of age weighed an average of 330.2 g (SD = 34.7, n = 12) and females weighed 279.8 g (SD = 41.1, n = 23) (ANOVA, P = 0.13). Males had significantly heavier bursae (0.646 g, SD = 0.301; versus 0.433 g, SD = 0.132; ANOVA, P = 0.003), and larger spleens (0.216 g, SD = 0.062, versus 0.154 g, SD = 0.053; ANOVA, P = 0.073) than females. However, the differences in body weight, tarsus length, bursa weight, and spleen weight were not affected by treatment. Because the treatment exposures were a few days only, differences in tarsal length would not normally be expected, and nor would body weight unless acute exposure caused dramatic weight loss. Spleen weight relative to body weight; 100× spleen weight/(body weight − spleen weight) was greatest in the spore-coated feed group. This exposure did present the greatest challenge in terms of mass and frequency of the fungus in the diet. This difference for the adjusted variable was weakly significant (control: 0.058, spore-coated feed: 0.063, infected insects: 0.053; ANOVA, P = 0.08).

Histopathology scores

The range of scores and median score for the organs of the 9-day-old and 46-day-old birds are shown in Tables 1 and 2.

Some of the scores were correlated among organs. In the 10-day-old birds, spleen scores and thymus scores were negatively correlated (R = −0.59, P = 0.005); cecum and proventriculus scores were positively correlated (R = 0.58, P = 0.005). For the 46-day-old birds, cecum

TABLE 1. Ratings (histology scores) of tissues of experimental groups of pheasants necropsied at age 10 days.

Treatment:	Control group		Spore-coated feed		Infected insect food	
Organ	Range	Median	Range	Median	Range	Median
Spleen	1–2	1	1–2	1	1–2	1
Bursa	1–2	1	1–2	2	1–2	2
Thymus	1–3	2	1–3	2	1–2	1–2
Lung	1–2	1	1–2	1	1–2	1
Crop	1	1	1–2	1	1	1
Proventriculus	1–2	1	1–3	1	1–2	1
Cecum	1–2	1	1–2	1	1	1
Liver	1–2	2	1–2	1	1–2	2

[a] The range of scores for the birds in the indicated group (n = 10 to 12 per range).

and thymus scores (R = 0.54, $P = 0.0008$), and liver and lung scores (R = 0.65, $P < 0.0001$), were positively correlated.

DISCUSSION

Dietary exposure of these young pheasants to *M. flavoviride* clearly had no adverse effect on behavior, appetite, or any of the other clinical variables assessed during the experimental period. The statistical correlations found among some of the tissues and their histology scores may be explained by the increased presence of antigen in the gastrointestinal tract (e.g., positive correlation between proventriculus and cecum), which would result in increased liver exposure to antigen through portal circulation, and may be provoking heterophil activity in the thymus. In birds, increased hepatic granulopoiesis with concurrent pulmonary granulopoiesis reflects a systemic response to antigenic stimulation in young gallinaceous birds.

In the bursa of Fabricius, the organ responsible for the genesis and differentiation of B lymphocytes in avian species, clusters of heterophils may commonly be found in the subepithelial tissue and along the margin of the follicles (Pope, 1996). At the day of hatch, this is a form of extramedullary granulocytopoiesis (the development and differentiation of leucocytes from the granulocytic cell line which occurs outside the bone marrow). In avian species, negative environmental factors such as nutritional, infectious or physical stress, result in glucocorticoid release and subsequent B and T lymphocyte death, or apoptosis. This may result in bursal and thymic atrophy, with increased numbers of apoptic cells scattered throughout the parenchyma, since these organs consist pri-

TABLE 2. Ratings of tissues of experimental groups of pheasants necropsied at age 46 days.

Treatment:	Control group		Spore-coated feed		Infected insect food	
Organ	Range	Median	Range	Median	Range	Median
Spleen	1–2	1	1–2	1	1–2	1
Spleen germinal centres	1–3	2	1–4	1–2	1–3	1
Bursa	1–2	1–2	1–3	1	1–3	1
Thymus	1–2	1	1–2	1	1–2	1
Lung	1–3	1–2	1–3	1–2	1–3	1
Crop	1	1	1–2	1	1–2	1
Proventriculus	1–2	2	1–2	1	1–2	1
Cecum	1–3	1–2	1–2	1	1–2	2
Liver	1–3	2	1–3	2	1–2	1
Liver glycogen	1–2	1	1–2	1	1–2	1

marily of lymphoid cell populations (Pope, 1996). If ingestion of the entomopathogenic fungi on the feed or on the infected grasshoppers were adversely affecting nutritional status, or if the fungi behaved as infectious agents provoking a strong immune response in the birds, increased bursal and thymic lymphocyte apoptosis would be expected.

The relative bursal weights of males compared with females was significantly greater when bursal weight was expressed relative to tarsal length, which is an indicator of body size, unaffected by the daily fluctuations of measurements such as body weight. A sex-related difference in bursal size has not been described in poultry (Nelson and Demas, 1996), and was not seen in house sparrows (*Passer domesticus*) being investigated for relationships between bursal size, sexual ornamentation, and immunocompetence (Moller et al., 1996). In spite of no statistical difference among exposure groups, the "contaminated feed" and "infected insect" exposed males had considerably larger bursae than either the control males or any of the females. This may be indicative of an increased immune effort being provoked by the increased dietary load of antigen in the males of the spore contaminated feed group. Because the change was evident only in males, it may be affected by circulating androgen levels which are reported to influence the immune response (Shuurs and Verheul, 1990). There was no histological evidence of hyperplastic change, which might have helped elucidate the nature of the observed bursal hypertrophy in the male pheasants.

Birds have nonencapsulated lymphoid tissue, with discrete lymph nodes being relatively rare (Banks, 1986). Diffuse lymphoid tissue normally develops in all avian tissues and organs, with germinal centres formation within these tissues being the normal response to antigenic stimulation (Eerola et al., 1987). Lymphoid nodules were quantified in the livers of these mycoinsecticide-exposed pheasants, and showed no evidence of increased germinal centre formation associated with higher numbers of nodules. The lack of germinal centre response in hepatic tissue provided further evidence that there was no major immunostimulation in these birds, as has been described by Pope (1991). The level of lymphoid activity in these birds' tissues was within the normal range for young, gallinaceous birds (C. Riddell, pers. comm.).

The spleen, which at hatch is a granulopoietic organ, rapidly transforms into a lymphocyte populated organ with approximately equal amounts of red and white pulp. In the white pulp, the germinal centre activity was quantified and found to have no correlation with treatment. General ingested and inhaled antigens produce low grade stimulation of the immune response in healthy birds with a normal immune response. In newly hatched chicks, germinal centres normally appear at about 10 days of age, but with intense antigenic stimulation, they may appear as early as 4 days post-hatch (Pope, 1996). Among the 9-day-old pheasants, five of the birds that had been exposed to the mycoinsecticide had premature development of splenic germinal centres, compared with no germinal centre formation in the control birds. This supported the concept of an increased challenge to their B lymphocyte–mediated immune response was stimulated by exposure to the mycoinsecticide. This quantifiable difference of immunoresponsiveness was modulated in the older birds, although those on mycoinsecticide-dressed feed did have the highest percentage of spleens with high numbers of germinal centres, and was the only group in which very strong germinal centre development was seen. Thus, direct, high-dose conidia exposure did appear to cause increased demands on the immune system, but this did not translate into increased disease in this non-target species.

As part of the search for alternatives to chemical insecticides, isolates of another fungus *Beauveria bassiana* have also been developed for potential control of grass-

hoppers and locusts. In Canada and the U.S., research and development of isolates of this fungus have been in progress since 1987 (Marcandier and Khatchatourians, 1987). Experimental studies with *B. bassiana* indicate the potential for damage to non-target insects, such as pollinators (Goettel and Johnson, 1992) and predaceous beetles (Pingal and Lewis, 1996). The mycotoxin oosporein, isolated from *B. bassiana* (Vining et al., 1962), is potentially toxic to birds, yet *B. bassiana* will not grow at avian body temperatures, which generally exceed 38 C. Althouse et al. (1997) found that American kestrels ingesting *B. bassiana* spores at 5×10^6 spores per g body weight (simulating encounter under typical field application rates) developed no gross pathological lesions or alterations of behavior. Little evidence of lethal impacts of entomopathogens on homeothermic vertebrates has been reported, although activity of the mycotoxin beauvericin on mammalian tissue has been described (Nakajyo et al., 1987). Among ectothermic reptiles and amphibians disease has been described. *Beauveria bassiana* has caused fatal lung infections in three captive giant tortoises (*Testudo elephantopus, T. gigantea elephantina*) at the Chicago Zoological Park (Georg, 1962), and in two captive American alligators (*Alligator mississipiensis*) (Fromtling et al., 1979). In general, mycotic infections are uncommon in reptiles (Jacobson, 1978), and seem to occur in captive animals subjected to inadequate management or environmental conditions.

Metarhizium flavoviride has been shown to be more active at higher temperatures than *B. bassiana* (Inglis et al., 1996), allowing *M. flavoviride* to be more effective in sunny conditions that stimulate basking by the target insects, and subsequent elevation in body temperature (Inglis at al., 1996).

Mycotoxins, the intermediary metabolites elaborated by many fungi, interfere with immune function by suppressing protein synthesis through various mechanisms, which impairs proliferation and maturation of lymphoid cells (Corrier, 1991). Grossly and histologically, this would be expressed as bursal, thymic and splenic atrophy due to increased lymphoid apoptosis. Such changes were not seen.

The degree of development of bronchus associated lymphoid tissue (BALT) is partially dependent upon antigen load. Therefore, increased occurrence of BALT would be anticipated if the entomopathogenic fungi in the birds' diets were acting as key respiratory antigens and stimulating an immune response (Jeurissen et al., 1994). Pulmonary pathology or BALT stimulation was not a feature in any of the exposure groups in this study. Heterophils or granulocytic leukocytes play a role in avian respiratory defense, similar to that of alveolar macrophages in mammals. Heterophils were present as scattered cells, or as larger focal accumulations, regardless of experimental exposure, with the most prominent collections being present in lungs of the control group, although this was not statistically significant.

Lesions were found in some birds, but not necessarily associated with fungal challenge, indicating that the pheasant chicks were being exposed to a moderate challenge from potentially disease-causing agents in their environment. The tissue changes described in these studies presumably represent a normal range of incidental pathological challenges in a group of young birds being raised in a captive, closed, indoor environment. There was no evidence that oral exposure to the entomopathogenic fungi had any negative or compromising effect on the disease resistance or productivity in these chicks. It must be pointed out that the total dose of fungal components consumed from birds eating two infected grasshoppers per day, may be greater or less than maximum exposures of wild birds feeding on infected insects during large scale mycoinsecticide control of acridid outbreaks, since food consumption is dependent upon the size of the birds relative to their prey items.

The results of this study show encouraging features regarding the safety of this effective pesticide in the search for alternatives to environmentally persistent and harsh chemical compounds. At risk are other vertebrate species which inhabit grassland ecosystems where locusts and grasshoppers are controlled. Further studies are in progress to examine the potential health impacts of this entomopathogen to poikilothermic vertebrates, in particular the desert lizard *Acanthodactylus dumerlii* (R. Peveling, University of Basel, Basel, Switzerland).

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LITERATURE CITED

AGRICULTURE AND AGRI-FOOD CANADA (AAFC). 1993. Special review of carbofuran insecticide: effects on avian fauna and value to agriculture. Discussion Document D93-02, AAFC, Ottawa, Ontario, Canada, 53 pp.

ALTHOUSE, C. M., B. E. PETERSEN, AND L. C. MCEWEN. 1997. Effects on young American kestrels (*Falco sparverius*) exposed to *Beauveria bassiana* bioinsecticide. Bulletin of Environmental Contamination and Toxicology 59: 507–512.

BANKS, W. J. 1986. Applied Veterinary Histology. 2nd Edition. Williams and Wilkins, Baltimore, Maryland, 583 pp.

BATEMAN, R. P., M. CAREY, D. MOORE, AND C. PRIOR. 1993. The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. Annals of Applied Biology 122: 145–152.

CORRIER, D. E. 1991. Mycotoxicosis: mechanisms of immunosuppression. Veterinary Immunology and Immunopathology 30: 73–87.

EEROLA, E., T. VEROMA, AND P. TOIVANEN. 1987. Special features in the structural organization of the avian lymphoid system. *In* Avian immunology: Basis and practice. Vol. 1. A. Toivanen and P. Toivanen (eds.). CRC Press, Boca Raton, Florida, pp. 9–21.

FROMTLING, R. A., S. D. KOSANKE, J. M. JENSEN, AND G. S. BULMER. 1979. Fatal *Beauveria bassiana* infection in a captive American alligator. Journal of the American Veterinary Medical Association 175: 934–936.

GEDDES, A. M. W. 1990. The relative importance of crop pests in sub-Saharan Africa. Natural Resources Institute Bulletin no. 36, Chatham, UK, 69 pp.

GOETTEL, M. S., AND D. L. JOHNSON. 1992. Environmental impact and safety of fungal biocontrol agents. *In* Biological Control of Locusts and Grasshoppers, C. Lomer and C. Prior (eds.). CAB International, Wallingford, UK, pp. 356–361.

GEORG, L. K., W. M. WILLIAMSON, E. B. TILDEN AND R. E. GETTY. 1962. Mycotic pulmonary disease of captive giant tortoises due to *Beauveria bassiana* and *Paecilomycea fumoso-roseus*. Sabouraudia 2: 80–86.

HEWITT, G. B., AND J. A. ONSAGER. 1982. A method for forecasting potential losses from grasshopper feeding on northern mixed prairie forages. Journal of Range Management 35: 53–57.

INGLIS, G. D., D. L. JOHNSON, K-J. CHENG, AND M. S. GOETTEL. 1997. Use of pathogen combinations to overcome constraints of temperature on entomopathogenic Hyphomycetes against grasshoppers. Biological Control 8: 143–152.

———, ———, AND M. S. GOETTEL. 1996. Effects of temperature and thermoregulation on mycosis by *Beauveria bassiana* in grasshoppers. Biological Control 7: 131–139.

JACOBSON, E., AND J. H. MILLER. 1978. Diseases of the repiratory system in reptiles. Veterinary Medicine/Small Animal Clinician 73: 1169–1175.

JAMES, P. C., AND G. A. FOX. 1987. Effects of some insecticides on productivity of burrowing owls. Blue Jay 45: 65–71.

JEURISSEN, S. H., L. VERVELDE, AND E. M. JANSE. 1994. Structure and function of lymphoid tissues of the chicken. Poultry Science Review 5: 183–207.

JOHNSON, D. L., O. OLFERT, M. DOLINSKI, AND L. HARRIS. 1996. GIS-based forecasts for management of grasshopper populations in Western Canada. Proceedings of the FAO International Symposium on Agricultural Pest Forecasting and Monitoring. *In* La Prevision et la Depistage des Ennémis des Cultures, G. Bourgeois and M. Guibord (eds.). Publié par la Réseau D'Avertissements Phytosanitaires du Québec. Québec City, Québec, pp. 109–112.

LOMER, C. J., C. PRIOR, AND C. KOOYMAN. 1997. Development of *Metarhizium* spp. for control of

locusts and grasshoppers. *In* Microbial Control of Grasshoppers and Locusts, M. S. Goettel and D. L. Johnson (eds.). Memoirs of the Entomological Society of Canada 171, Ottawa, Ontario, pp. 265–286.

MARCANDIER, S. AND G. G. KHACHATOURIANS. 1987. Susceptibility of the migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae), to *Beauveria bassiana* (Bals.) Vuilemin (Hyphomycete): influence of relative humidity. Canadian Entomology 119: 901–907.

MARTIN, P. A., D. L. JOHNSON, AND D. J. FORSYTH. 1995. Effects of grasshopper control insecticides on survival and brain acetylcholinesterase of pheasant (*Phasianus colchicus*) chicks. Environmental Toxicology and Chemistry 15: 518–524.

MOLLER, A. P., R. T. KIMBALL, AND J. ERRITZOE. 1996. Sexual ornamentation, condition and immune defense in the house sparrow *Passer domesticus.* Behavioural Ecology and Sociobiology 39: 317–322.

NAKAJYO, S., K. MATSUOKA, T. KITAYAMA, Y. YAMAMURA, K. SHIMIZU, M. KIMURA, AND N. URAKAWA. 1987. Inhibitory effect of beauvericin on a high K^+-induced tonic contraction in guinea-pig taenia coli. Japanese Journal of Pharmacology 45: 317–325.

NELSON, R. J., AND G. E. DEMAS. 1996. Seasonal changes in immune function. The Quarterly Review of Biology 71: 511–548.

PEST MANAGEMENT REGULATORY AGENCY (PMRA). 1995. Carbofuran. Decision document E95-05, Health Canada, Nepean, Ontario, Canada., 14 pp.

PINGAL, R. L., AND L. C. LEWIS. 1996. The fungus *Beauveria bassiana* (Balsamo) Vuillemin in a corn ecosystem: its effect on the insect predator *Coleomegilla maculata* De Geer. Biological Control 6: 137–141.

POPE, C. R. 1991. Pathology of lymphoid organs with emphasis on immunosuppression. Veterinary Immunology and Immunopathology 30: 31–44.

———. 1996. Lymphoid System. *In* Avian Histopathology, 2nd Edition. C. Riddell (ed.). American Association of Avian Pathologists, Tallahassee, Florida, pp. 17–44.

PRIOR, C., C. LOMER, H. HERREN, A. PARAÏSO, C. KOOYMAN, AND J. J. SMIT. 1992. The IIBC/IITA/DFPV collaborative research programme on the biological control of locusts and grasshoppers. *In* Biological control of locusts and grasshoppers. C. J. Lomer and C. Prior (eds.), CAB International, Wallingford, UK, pp. 8–18.

RITCHIE, J. M., AND H. DOBSON. 1995. Desert locust control operations and their environmental impact. Bulletin No. 67, Natural Resources Institute, Overseas Development Administration, Chatham, UK., 42 pp.

SAS INSTITUTE. 1990. Statistical analysis system (SAS) user's guide: Statistics. SAS Institute Incorporated, Cary, North Carolina, 1,686 pp.

SCOTT, P. M., H. L. TRENHOLM, AND M. D. SUTTON (EDS.). 1985. Mycotoxins: A Canadian Perspective. NRCC Publication #22848, Ottawa, Ontario, Canada, 185 pp.

SEMALULU, S. S., J. M. MACPHERSON, H. B. SCHIEFER, AND G. G. KHACHATOURIANS. 1992. Pathogenicity of *Beauveria bassiana* in mice. Journal of Veterinary Medicine 39: 81–87.

SHOWLER, A. T. 1995. Locust (Orthoptera: Acrididae) outbreak in Africa and Asia, 1992–1994: an overview. American Entomologist 41: 179–185.

———, AND C. S. POTTER. 1991. Synopsis of the 1986–1989 desert locust (Orthoptera: Acrididae) plague and the concept of strategic control. American Entomologist 37: 106–110.

SHUURS, A. H., AND H. A. VERHEUL. 1990. Effects of gender and sex steroids on the immune response. Journal of Steroid Biochemistry 35: 157–172.

VEEN, K. H., AND P. FERRON. 1966. A selective medium for the isolation of *Beauveria tenella* and of *Metarrhizium anisopliae.* Journal of Invertebrate Pathology 8: 268–269.

VINING, L. C., W. J. KELLEHER AND A. E. SCHWARTING. 1962. Oosporein production by a strain of *Beauveria bassiana* originally identified as *Amanita muscaria.* Canadian Journal of Microbiology 8: 931–933.

ZACCAGNINI, M. E., A. MUSTACCIOLO, V. LICHTSCHEIN, S. CANAVELLI, C. SALTO, AND C. GONZALEZ. 1998. Pesticide impacts on Swainson's hawks and the development of a national ecotoxicological monitoring program in Argentina. *In* Proceedings of the 19th Annual Meeting, Abstract 602. Society of Environmental Toxicology and Chemistry, Pensacola, Florida, p. 127.

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