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EFFECTS OF GRASSHOPPER-CONTROL INSECTICIDES ON SURVIVAL AND BRAIN ACETYLCHOLINESTERASE OF PHEASANT (*PHASIANUS COLCHICUS*) CHICKS

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Abstract—Ring-necked pheasant (*Phasianus colchicus*) chicks were exposed via contact and/or ingestion to formulations of three insecticides (Lorsban 4E, chlorpyrifos; Cygon 480E, dimethoate; and Furadan 480F, carbofuran) applied to pasture plots at one and four times the rate recommended for control of grasshoppers (Orthoptera: Acrididae) in prairie Canada. Chicks (3 d old) were exposed for 48 h in pens with the sprayed vegetation and were fed either unsprayed grasshoppers or grasshoppers sprayed at the same rates as the vegetation. Control groups were exposed to unsprayed vegetation and received unsprayed grasshoppers. Three replicates were conducted throughout June and early July 1992.

Although some signs of acetylcholinesterase (AChE) depression were observed in chicks exposed to insecticides, there was no difference in the number of mortalities among treatment and control groups. Chicks that died during the exposure period gained significantly less weight than survivors in all groups. Among surviving chicks, weight gains of those exposed to the high- rate Furadan treatment consuming sprayed food were significantly lower than those of controls (1.8 vs. 6.6 g/d). Brain AChE activity was lower overall in surviving chicks than in those that died; it was not significantly reduced among chicks that died in any treatment group. Overall, survivors of Furadan- exposed treatment regimes had lower AChE activity than those of Cygon and Lorsban treatments; birds in high-spray-rate treatments of all insecticides had lower AChE activity than those in all low-spray-rate treatments; and birds in all treatments consuming sprayed grasshoppers had lower AChE activity than those fed unsprayed grasshoppers. Food consumption was not affected by any treatment.

Keywords—Acetylcholinesterase Carbofuran Chlorpyrifos Dimethoate Ringed-necked pheasants

INTRODUCTION

In years of severe grasshopper infestations, large areas of the grassland and cereal cropland in western Canada are sprayed with insecticides. In 1985, between 3.7 and 4.7 million ha across the three prairie provinces were sprayed at least once; in 1986, between 2.8 and 3.2 million ha were sprayed in Saskatchewan alone [1,2]. Up to 20% of the arable land area in Alberta has been infested since 1983, and farmers spent $12 million on grasshopper-control insecticides during 1985 and 1986 [3]. Because grasshopper eggs overwinter in uncultivated areas such as pastures, rangeland, hayfields, and roadsides, these areas are preferentially sprayed as the nymphs emerge in the spring. Such areas frequently represent the only remnants of wildlife habitat available in the intensively farmed prairie landscape. Managers of cooperative and private grazing land attempt to control grasshoppers on their property under pressure from adjacent crop farmers and in an effort to limit the reduction in grazing potential and forage production on pasture vegetation or hayfields. Thus, wildlife is at risk of exposure to grasshopper-control insecticides over widespread areas during years of grasshopper infestation and in more localized locations between outbreaks.

Liquid formulations of the cholinesterase-inhibiting organophosphorus and carbamate compounds are the insecticides most commonly used in recent grasshopper-control programs. Carbofuran typically accounts for the majority of the insecticide market. This insecticide has been reported to cause death in waterfowl feeding in sprayed vegetation on wintering grounds [4] and in gulls (*Larus californicus*) gorging on grasshoppers in sprayed fields in Saskatchewan [5]. In 1985 and 1986, dimethoate was implicated in the deaths of large numbers of sage grouse (*Centrocercus urophasianus*) in alfalfa fields in Idaho [6]. Such reports suggest that these two insecticides may present a more widespread hazard to grassland birds.

Previous studies have indicated that mallard (*Anas platyrhynchos*) ducklings walking 200 to 300 m through upland vegetation sprayed with either carbofuran, dimethoate, or chlorpyrifos were essentially unaffected unless feeding occurred *en route* [7,8, P.A. Martin and D.J. Forsyth, unpublished data]. Juvenile waterfowl spend very little time feeding in the uplands, whereas galliform species, which are permanent residents of upland habitat, are probably at greater risk of pesticide effects.

Sharp-tailed grouse (*Tympanuchus phasianellus*), ring-necked pheasants (*Phasianus colchicus*), and gray partridge (*Perdix perdix*) use pastures, hayfields, field margins, and roadsides extensively during the brood-rearing period [9–11]. Spraying for grasshoppers in these areas occurs from late May through late July, coinciding with the brood-rearing period. Very young chicks may be particularly susceptible to direct toxic effects of anticholinesterase insecticides because of their small body size. They may also be more sensitive to insecticides so that a smaller dose relative to body weight could cause intoxication, although the opposite has also been demonstrated [12,13]. During their first 3 to 4 weeks of life, juvenile sharp-tailed grouse, gray partridge, and pheasants consume large quantities of arthropods [9,11,14], a food source that may be contaminated by insecticides following spray applications. Cholinesterase-inhibiting insecticides interfere with thermoregulation in birds and may decrease the ability of young chicks, which have incompletely developed thermoregulatory systems, to maintain body temperature during periods of cold, wet weather [15,16].

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The objectives of this study were to assess the impact of three insecticides commonly used for grasshopper control in Canada on the survival and cholinesterase activity of ring-necked pheasant chicks and to compare the relative contributions to toxicity of exposure through contaminated insect prey versus contaminated vegetation.

METHODS

Study site

The field/pen study was conducted within a 64-ha pasture of native grassland belonging to the Brooks Horticultural Research Station, Alberta Ministry of Agriculture, Food and Rural Development, located 10 km south of Brooks, Alberta, Canada, in the mixed-grass prairie ecoregion. Vegetation was predominantly blue grama grass (*Bouteloua gracilis*) and sage (*Artemesia cana* and *A. frigida*). The pasture was ungrazed and unburned.

Study animals

Newly hatched female pheasant chicks were obtained from the Brooks Wildlife Centre (Alberta Forestry, Lands, and Wildlife, Brooks, AB, Canada) and kept in indoor brooder pens in groups of 80 to 100 for 3 d. They were provided with concentrated turkey starter ration (Newlife Feeds, Lethbridge, AB, Canada, 29% protein) and water, and the temperature was maintained at 35 to 38°C. At 3 d old, chicks were randomly assigned to treatment groups (six per group), weighed (± 0.1 g) using a 50-g spring scale (Pesola, Switzerland), measured (tarsus length) using Dial metric calipers (± 1.0 mm), and marked with numbered squares of colored nylon tape glued to feathers on their backs between the wings. The combination of tape color and number uniquely identified each bird.

Experimental procedure

Between 0500 and 0700 h on day 4, field plots were sprayed using a bicycle-mounted pressurized sprayer designed to deliver pesticide in the same manner as typical tractor-mounted sprayers. Pressure was 275 kPa, and the spray volume was 110 L/ha. Insecticides included Furadan 480F (carbofuran flowable, 480 g a.i./L, Miles Inc., Mississauga, ON, Canada), Cygon 480E (dimethoate emulsifiable, 480 g a.i./L, Cyanamid Ltd., Markham, ON, Canada), and Lorsban 4E (chlorpyrifos emulsifiable, 400 g a.i./L, DowElanco, Newmarket, ON, Canada). Insecticides were applied at one and four times the highest rates recommended for grasshopper control in western Canada [17]. These rates were chosen to represent what was assumed to be typical field scenarios and to encompass the possibility of accidental multiple-swath overlaps, tank-mixing errors, and higher rates used for other crops. Rates were as follows: (a) Furadan, 132 and 528 g a.i./ha; (b) Cygon, 213 and 852 g a.i./ha; and (c) Lorsban, 279 and 1,116 g a.i./ha. Formulated products were mixed with tap water (pH 7.9) immediately before spray application. Spray deposit was measured by placing two rows of four filter papers (9-mm diameter) on the soil surface at intervals across the width of the swaths at canopy height (15 cm) for each application; filter papers within each row were pooled for residue analysis. Each spray swath was 2 m wide and 10 m long, which was sufficient to accommodate two pens (1 m wide × 2 m long × 1 m high) separated by 4 m. Pens were paired in plots, with one housing chicks fed uncontaminated food and the other housing chicks fed grasshoppers that had been sprayed at the same rate as the plot. This procedure allowed for comparison of relative effects of contaminated food plus vegetation versus contaminated vegetation alone for each insecticide at the two rates of application.

Immediately after all plots had been sprayed, pens were erected over the sprayed grass cover. Pens were constructed of 2.5-cm mesh chicken wire secured to metal corner supports covered with 5-cm mesh chicken wire. Each was equipped with a wooden brooder box (30 × 60 × 30 cm) placed in one corner and heated with a red incandescent 40-watt lightbulb. Food dishes were placed on small squares of plywood to reduce contamination from sprayed vegetation and to catch spilled food items in order to facilitate measurement of food consumption. Dishes were kept at the entrance of the boxes during the day to keep food sheltered from wind and sunlight. Water dishes were placed outside near the brooder box during the day but were moved inside at night. Dishes were checked periodically, and preweighed amounts of the appropriately treated food was added as needed to maintain a constant supply. Uneaten food was removed and weighed. Total food consumption per pen was tallied over the 48-h exposure period, and the amount of food eaten per chick-day was calculated for each pen.

Chicks were fed an exclusive diet of grasshoppers throughout the 48-h exposure period. Three species of grasshoppers were collected from the field and sprayed with field-equivalent rates of the three insecticides. The species used were the clear-winged grasshopper (*Camnula pellucida*), the two-striped grasshopper (*Melanoplus bivittatus*), and the lesser migratory grasshopper (*Melanoplus sanguinipes*). Insecticide solutions were prepared volumetrically with tap water (pH 7.5) and sprayed with a laboratory spray chamber (Innovative Equipment, Rogers Engineering, Saskatoon, SK, Canada). The spray volume for all insecticide treatments was equivalent to a field application volume of 80 L/ha. The insecticides were sprayed through a single T-Jet SS8001E nozzle (100-mesh screen) at 201 to 204 kPa. Ambient spray chamber temperature during spraying was 22 to 24°C. To maintain equal volume of output among treatments, the selected nozzle track velocity depended on the insecticide product and concentration and ranged from 1.1 to 1.8 m/s. Spray droplet size and concentration were monitored every fourth pass of the spray nozzle with water-sensitive paper and with filter papers for residue analysis. Droplets in six randomly selected 0.5 × 0.5-cm areas per treatment were counted and measured under a dissecting microscope to ensure equal coverage for all insecticide treatments. Grasshoppers were lightly anesthetized by directing a stream of CO_2 gas into each container for 3 s. The resulting 3-min period of anesthesia provided enough time for spray application without recovery and movement of the insects. Anesthetized grasshoppers were arranged 62 cm below the nozzle and sprayed once. Grasshoppers in the control groups were anesthetized, handled, and caged in the same way as the treated grasshoppers but were not sprayed. Sprayed and unsprayed insects were frozen and transported to the field for feeding to chicks in the cages. The application sequence was replicated according to the number of cages to be fed.

Overall there were 13 treatment groups, including controls (three insecticides, two application rates, and two food sources, i.e., sprayed or unsprayed). Three control pens were included in each replicate, and three complete replicate trials were conducted.

Chicks were brought to the field after all the pens were erected (at 1000 to 1200 h) and were placed in the appropriate pens. Food was provided immediately. At approx. 2200 h each evening, chicks were locked inside the brooder boxes to ensure that they stayed warm during the night. They were released and

given fresh food and water between 0630 and 0800 h both subsequent mornings. Birds were observed from 2 to 5 m away at 2- to 4-h intervals during daylight hours. Moribund birds were monitored closely until death or recovery. If death occurred, chicks were chilled on ice immediately and taken to the laboratory freezer (−40°C) within 1 h. Birds found dead at the first morning check were chilled immediately. Surviving chicks were sacrificed using carbon monoxide poisoning at the end of exposure. The brains of all chicks were excised and frozen in liquid nitrogen for subsequent analysis of acetylcholinesterase (AChE) activity. The total exposure period was 48 h.

Acetylcholinesterase analysis

Analysis for AChE activity was conducted at the National Wildlife Research Centre (Hull, PQ, Canada). Brains were homogenized in chilled Tris buffer (pH 8.0) using glass tissue grinders. Homogenates were assayed colorimetrically at 410 nm at 25°C using acetylthiocholine iodide (Sigma Chemical Co., St. Louis, MO, USA) as the enzyme substrate [18,19]. Activity was expressed as micromole substrate hydrolyzed per minute per gram brain tissue (wet weight).

Insecticide residue analysis

Analyses were conducted by EnviroTest Laboratories (Edmonton, AB, Canada). Filter-paper field samples were shaken with 40 ml of dichloromethane. Grasshoppers were extracted twice by polytroning with 1:1 acetonitrile : hexane. Extracts were combined and the acetonitrile drained through regular sodium sulphate. The remaining hexane was partitioned with more acetonitrile, which was then reduced to a final volume for analysis. Sample aliquots were taken for insecticide analysis by gas chromatography/mass spectrometry using a Hewlett-Packard (HP) 5890 GC with an HP 1701 column (30 m × 0.25 mm i.d.) coupled with an HP 5975 mass selective detector. Detection limits for filter-paper samples were 1, 50, and 10 μg for carbofuran, dimethoate, and chlorpyrifos, respectively; for grasshoppers they were 0.01, 0.5, and 0.1 μg/g for the three insecticides, respectively. Recoveries for carbofuran, dimethoate, and chlorpyrifos were 95, 74, and 107% from spiked filter-paper samples and 103, 73, and 97% from spiked grasshopper homogenate. Reported values have been corrected for recovery efficiency.

Statistical analyses

Statistical analyses were conducted using the CATMOD and GLM procedures of SAS® [20]. Differences in mortality among the 13 treatment groups pooled across all replicates were tested using G-tests. An initial analysis of variance (ANOVA) using a split–split plot design was used to examine the overall effects of insecticide, application rate, and food contamination regime on daily growth rate (change in weight and tarsus length) and brain AChE activity. Insecticide was the main plot factor; rate, the split plot factor; and food, the split–split plot factor. To test the main plot factor, the insecticide × replicate mean square was used as the error term; to test the rate and rate × insecticide factors, the replicate × rate × insecticide mean square was used as the error term. The effect of food and its interactions were tested with the model error mean square. Control groups were omitted from this analysis. Orthogonal contrasts were conducted to test a priori questions of specific interest [21]. We subsequently conducted a nested ANOVA on each pesticide separately, including pooled control groups in the analyses for comparisons, using the treatment × replicate mean square as the error term in testing the effects of treatment. Mortalities and survivors were analyzed separately. We determined differences between treatment and control means using Tukey's honestly significant difference tests (PROC GLM).

Table 1.Spray deposit and chemical residues in grasshoppers

Insecticide	Nominal rate (g a.i./ha)	Spray deposit (g a.i./ha) n[a]	Mean	SE	% Nominal	Concn. (μg/g) n[b]	No. 1	No. 2
Lorsban	279	3	296	76	106	2	16	19
	1,116	3	1,160	269	104	2	73	83
Cygon	213	3	218	122	102	2	4.0	4.1
	852	3	1,116	629	131	2	13.4	16.4
Furadan	132	3	119	69	90	2	6.8	6.1
	528	3	690	398	131	2	35	38

[a]One composite filter-paper (four per pool) sample per replicate trial.
[b]Pooled grasshopper samples from two track sprayer applications.

RESULTS

Spray deposit and grasshopper residues

The mean spray deposit over the three replicate trials was slightly higher than 100% of the nominal rate for all applications (Table 1). However, deposits in the first two replicates were between 40 and 100% of nominal for most applications, while those in the third replicate ranged from 120 to 200% of nominal. Residue concentrations showed good consistency between the two pooled grasshopper samples prepared in the track sprayer (Table 1).

Mortality

Of 270 pheasant chicks monitored during the experiment, a total of 37 died and 17 displayed symptoms of cholinesterase inhibition. The number of chicks that died or showed symptoms during insecticide exposure did not differ among treatment groups (Table 2). In replicate 1, almost all mortalities occurred during the first night; chicks were dead in the brooder boxes at the first morning check. Three chicks in the high-rate Lorsban group fed contaminated food died 2 h after the first morning check following a feeding bout. Symptoms of toxicity were not observed. In replicate 2, the only two chicks that died were in the low-rate Furadan group receiving uncontaminated food. One chick died about 24 h after initial exposure and 3 h after release from the brooder box; the second bird was dead upon release from the brooder box on the second morning. The third replicate had the highest levels of mortality overall; all but two deaths occurred during the second night of exposure, probably a result of the unusually cold, damp weather. The two earlier deaths, one in the low- rate Lorsban group receiving uncontaminated food and one in the low-rate Cygon group fed sprayed food, occurred at about 24 h exposure, 2 to 3 h after release from the brooder boxes. The chick in the Cygon group underwent spasms and paralysis prior to death.

Growth and food consumption

In the split–split plot ANOVA, the main factors of insecticide, spray rate, or food contamination did not affect tarsal growth or weight gain, although weight gain was significantly affected by the interaction of the three factors ($p < 0.05$).

Large differences were observed in the rates of weight gain between birds that survived and those that died, pooled across

Table 2.Number of pheasant chicks that died or exhibited symptoms of cholinesterase inhibition during 48 h of exposure to Lorsban, Cygon, or Furadan in three replicate trials of 12 treatment regimes plus untreated controls

Insecticide	Nominal rate (g a.i./ha)	Food[a]	Replicate 1			Replicate 2			Replicate 3			Total		
			n	Dead	Symptoms[b]	*n*	Dead	Symptoms[b]	*n*	Dead	Symptoms[b]	*n*	Dead	Symptoms
Lorsban	279	Unsprayed	6	0	3	6	0	0	6	3	3	18	3	3
		Sprayed	6	1	0	6	0	0	6	0	0	18	2	0
	1,116	Unsprayed	6	1	0	6	0	0	6	2	1	18	3	1
		Sprayed	6	3	0	6	0	0	6	0	0	18	3	0
Cygon	213	Unsprayed	6	2	0	6	0	0	6	1	0	18	4	0
		Sprayed	6	0	1	6	0	0	6	2	2	18	3	2
	852	Unsprayed	6	2	0	6	0	2	6	2	2	18	4	2
		Sprayed	6	1	0	6	0	0	6	2	0	18	3	0
Furadan	132	Unsprayed	6	1	0	6	2	0	6	0	0	18	4	0
		Sprayed	6	0	1	6	0	0	6	1	0	18	1	0
	264	Unsprayed	6	0	0	6	0	3	6	1	3	18	1	3
		Sprayed	6	0	1	6	0	2	6	2	6	18	2	6
Control			18	1	0	18	0	0	18	3	0	54	4	0

[a]Food regimes consisted of either unsprayed grasshoppers (unsprayed) or grasshoppers sprayed at the same rate as the vegetation for that treatment (sprayed).
[b]Symptoms included lethargy, persistent head twitching and gulping, and spasms.

all treatment groups, including controls (Table 3) ($p < 0.0001$); overall mean changes in weight were 4.8 and −0.2 g/d, respectively. Rate of tarsal growth showed a similar difference ($p < 0.0001$); overall gain in tarsus length was 1.3 and 0.6 mm/d for survivors and mortalities, respectively. Therefore, when nested ANOVAs were conducted on individual insecticides, we analyzed survivors and mortalities separately.

Mean rate of change in body weight of survivors of all insecticide treatments was lower than that of controls. Nevertheless, although the overall treatment effect was significant in the groups exposed to Cygon and Furadan compared to controls ($p < 0.006$), the only treatment mean that differed significantly from that of the control group was the high-rate Furadan group consuming sprayed grasshoppers (1.8 vs. 6.6 g/d, Table 3). Birds exposed to both the high-rate Lorsban and the low-rate Cygon receiving unsprayed grasshoppers had mean rates of growth of less than 50% that of controls (2.9 and 3.2 vs. 6.61 g/d, respectively, Table 3), although differences were not significant. Rate of tarsal growth in surviving chicks was unaffected by any of the insecticide treatment regimes in comparison to controls ($p > 0.40$). Neither growth parameter was affected by insecticide treatments in chicks that died during exposure ($p > 0.10$).

No significant differences in food consumption were observed among any of the treatment regimes and the control group (Table 4).

Table 3.Mean daily changes in body weight (g) of pheasant chicks that survived or died during a 48-h exposure to one of three insecticides compared to untreated controls

Insecticide	Nominal rate (g a.i./ha)	Food[a]	Survivors			Mortalities		
			n	Mean	SE	*n*	Mean	SE
Lorsban	279	Unsprayed	15	4.9	1.3	3	−0.5	0.2
		Sprayed	17	4.7	0.8	1	−2.5	—
	1,116	Unsprayed	14	2.9	0.9	3	−1.4	0.5
		Sprayed	15	4.1	1.2	3	3.2	0.5
Cygon	213	Unsprayed	14	3.2	0.8	3	0.1	0.4
		Sprayed	16	5.0	1.3	2	−2.8	2.0
	852	Unsprayed	14	4.6	1.2	4	1.1	1.1
		Sprayed	14	4.4	1.0	3	0.2	0.3
Furadan	132	Unsprayed	15	4.8	1.4	3	−1.4	2.1
		Sprayed	17	5.4	1.3	1	0.2	—
	528	Unsprayed	17	5.8	1.3	1	−0.7	—
		Sprayed	16	1.8[b]	1.0	2	−1.1	0.4
Control			48	6.6	0.7	4	−0.5	0.8

[a]Food regimes consisted of either unsprayed grasshoppers (unsprayed) or grasshoppers sprayed at the same rate as the vegetation for that treatment (sprayed)
[b]Mean significantly different from control mean (nested ANOVAs within insecticide, compared to pooled controls in each analysis, Tukey's honestly significant difference test, $p < 0.05$.

Brain acetylcholinesterase

All three main factors in the split–split plot ANOVA, insecticide, rate, and food contamination, significantly affected AChE activity of pheasant chicks (p values = 0.03, 0.0005, and < 0.0001, respectively). Overall mean AChE activity of Furadan-exposed groups was less than the means for groups ex-

Table 4.Food consumed per chick per day during exposure to Lorsban Cygon, or Furadan treatments

Insecticide	Nominal rate (g a.i./ha)	Food[a]	Grasshoppers eaten (g wet weight)		
			n	Mean[b]	SE
Lorsban	279	Unsprayed	3	13.2	1.0
		Sprayed	3	15.4	0.1
	1,116	Unsprayed	3	14.4	2.5
		Sprayed	3	13.0	0.8
Cygon	213	Unsprayed	3	13.4	2.5
		Sprayed	3	13.9	0.8
	852	Unsprayed	3	14.2	2.4
		Sprayed	3	12.2	2.6
Furadan	132	Unsprayed	3	15.1	1.0
		Sprayed	3	10.9	4.8
	528	Unsprayed	3	15.4	1.9
		Sprayed	3	12.4	1.1
Control			9	15.5	1.0

[a]Food regimes consisted of either unsprayed grasshoppers (unsprayed) or grasshoppers sprayed at the same rate as the vegetation for that treatment (sprayed)
[b]Mean of three replicate trials. Food consumption was measured on a per-pen basis, not a per-chick basis, but corrected for number of chicks per pen.

Table 5.Brain acethylcholinesterase (AChE) activity (as % control) of pheasant chicks either dying during or surviving a 48-h exposure to one of three insecticides

Insecticide	Nominal rate (g a.i./ha)	Food[a]	Survivors[b]			Mortalities[c]		
			n	Mean	SE	*n*	Mean	SE
Lorsban	279	Unsprayed	15	97.7	3.2	3	117.3	1.5
		Sprayed	17	94.5	3.7	1	117.6	—
	1,116	Unsprayed	14	89.4	5.3	3	93.9	13.8
		Sprayed	15	86.2	6.1	3	107.4	4.6
Cygon	213	Unsprayed	14	102.6	2.2	3	97.2	15.8
		Sprayed	16	90.8[d]	2.4	2	111.9	6.9
	852	Unsprayed	14	94.8	1.8	4	91.1	13.6
		Sprayed	14	70.1[d]	5.5	3	91.7	10.7
Furadan	132	Unsprayed	15	97.0	2.5	3	101.7	8.7
		Sprayed	17	87.6[d]	3.0	1	109.4	—
	528	Unsprayed	17	77.0[d]	3.2	1	96.4	—
		Sprayed	16	64.1[d]	6.4	2	109.4	0.7

[a]Food regimes consisted of either unsprayed grasshoppers (unsprayed) or grasshoppers sprayed at the same rate as the vegetation for that treatment (sprayed).
[b]Mean brain AChE activity of untreated (control) survivors ($n = 48$) was 34.8 (SE = 0.5) μmol acetylthiocholine hydrolyzed/min/g brain tissue.
[c]Mean brain AChE activity of untreated (control) mortalities ($n = 4$) was 33.3 (SE = 5.5) μmol acetylthiocholine hydrolyzed/min/g brain tissue.
[d]Means significantly different from control group mean (nested ANOVAs within insecticide, compared to pooled controls in each analysis, Tukey's honestly significant difference test, $p < 0.05$).

posed to Cygon or Lorsban; mean AChE activity of birds exposed to high spray rates was lower than that of those exposed to low spray rates; and birds consuming sprayed food had lower AChE activity than those fed unsprayed food. A planned orthogonal contrast showed overall mean brain AChE activity of birds in the Furadan treatments was slightly lower than that of birds in all Cygon and Lorsban treatments together ($p = 0.014$, means of 29.0 vs. 31.5 μmol/min/g).

Overall, birds that died had significantly higher brain AChE activity than those that survived (33.9 vs. 31.5 μmol/min/g, $p = 0.03$), so further analysis by pesticide was conducted on these two groups separately. Treatment had a significant effect in birds surviving the Cygon and Furadan regimes ($p < 0.001$). Brain AChE activity was lower in birds surviving exposure to Cygon-sprayed grasshoppers at both low and high rates than in controls (Table 5). Birds fed Furadan-treated grasshoppers at both spray rates, as well as those exposed to the high-rate vegetation consuming untreated food, exhibited significantly depressed brain AChE activity (Table 5). Mean brain AChE activity of birds fed unsprayed grasshoppers and exposed to vegetation treated with high-rate Furadan was lower than that of birds eating food treated at the lower rate.

Brain AChE activity of birds that died during exposure to any of the insecticide regimes did not differ significantly from that of controls (Table 5).

DISCUSSION

Depositions of the three grasshopper-control insecticides measured in our sprayed plots were somewhat higher than those detected in aerial applications reported to have ranged from 28 to 100% of target rate [22–24]. This variation in pesticide deposition provides information for the worst-case scenario at a given spray rate. Grasshopper concentrations were consistent between the two spraying events used in their preparation. In a previous study, grasshoppers collected following aerial applications of Furadan at 134 g a.i./ha to two pastures contained mean carbofuran concentrations of 2.2 and 3.1 μg/g wet weight for live grasshoppers and 3.9 μg/g for dead grasshoppers [25]. Grasshoppers in the gullets of California gulls that died on agricultural land recently sprayed with Furadan contained 4.2 to 7.2 μg/g carbofuran [5]. Thus, concentrations in our laboratory-sprayed grasshoppers appear to be representative of concentrations in insects available to birds foraging in the field.

Despite the low levels of mortality, signs of anticholinesterase toxicity were observed in 17 of 216 pesticide-exposed pheasant chicks (7.9%); six of these occurred in the pen treated with the high-rate Furadan and receiving sprayed food. Most birds recovered within a few hours of the onset of symptoms and appeared to suffer no prolonged effects. Nevertheless, this period of sickness may have serious consequences for broods in the wild. Chicks of precocial species requiring parental brooding and direction may be abandoned by their parents during a spell of incapacitation, thereby reducing their chances of survival. Martin and Forsyth [8] found that mallard hens moving overland with their broods would wait only briefly (minutes) for a Furadan-intoxicated duckling and would soon abandoned it.

Trends of reduced weight gain also were observed in birds exposed to insecticide-sprayed grass, particularly grass treated at the high spray rates, suggesting some longer-term impacts on pheasant chick growth. In particular, birds exposed to the grass treated at the high spray rate of Furadan and fed sprayed grasshoppers grew more slowly than control birds. However, 18-week-old turkeys living in range pens where grass and soil had been sprayed with much higher levels of chlorpyrifos than used in the present study (2 and 4 kg a.i./ha) for 28 d achieved the same body weight as control birds [W.S. McGregor and R.W. Swart, unpublished data].

All chicks that died during the exposure period, including controls, gained weight significantly more slowly than surviving chicks, and most actually lost weight. Insecticide-exposed birds experienced no greater loss in weight than did control birds, however, indicating minimal additional impact of insecticides as a factor contributing to weight loss. Brain AChE activity was not depressed in exposed chicks that died, further suggesting an insignificant role played by acute insecticide toxicity in this study. Some birds were found dead at the first check in the morning; thus, time of mortality was unknown. It is possible, therefore, that some postmortem reactivation of brain AChE activity may have occurred. Brain AChE activity of Japanese quail administered a sublethal dose of carbofuran (1 mg/kg) and euthanized 1 h later had reactivated to control levels 24 h postmortem when held at 25°C [26]. In contrast, a similar dose of the organophosphorus compound dicrotophos caused continued decline in AChE activity for 72 h postmortem [26]. At lethal dosages of carbofuran, however, despite slight postmortem reactivation, brain AChE activity was still significantly lower than in controls 72 h after death; activity of dicrotophos-dosed birds continued to decline during this period [26]. Brain AChE activity was equally depressed immediately and 7 d postmortem in Japanese quail (*Coturnix japonica*) lethally dosed with dimethoate [27]. If brain AChE activity of pheasant chicks in the present study had been depressed to the levels usually associated with death (<50% of control activity [28]), significant depression should still have been detectable despite the delay in brain removal and cold-storage after death. It is likely, therefore, that

these birds died because of starvation or hypothermia exacerbated by the stresses of the outdoor penning situation despite the provision of brooder boxes, which would explain the deaths in control birds as well. Although anticholinesterase insecticides have been shown to reduce thermoregulatory capability in the young of precocial species [15,29], this was probably not a factor in the deaths of our insecticide-exposed chicks because they suffered no greater mortality than did control chicks. Significant reductions in the thermoregulatory abilities of young ducklings occurred only when brain AChE activity was inhibited by greater than 50% [15].

Considerable variability in the rates of weight gain among birds within treatment groups was observed. This variability may have indicated greater sensitivity of some individuals to the taste or toxic effects of the insecticide. Some birds may have avoided the food because of either a taste aversion or a conditioned aversion caused by associating food with anticholinesterase symptoms [30,31]. Other individuals within a group must have been able to consume adequate quantities of food to maintain normal growth rates while avoiding a lethal dose. This is further evidenced by the fact that, on a pen basis, chicks in treatment groups receiving insecticide-contaminated food sources did not consume less food than controls. Half of a group of northern bobwhite (*Colinus virginianus*) chicks exposed to food containing carbofuran at 159 μg/g for 5 d reduced their consumption of treated and clean food combined by 25% relative to control birds [32]. Pheasant hens reduced consumption by 28% when provided with Furadan-treated food (carbofuran at 50 μg/g) and by 90% when provided with Lorsban-treated food (chlorpyrifos at 2,200 μg/g) alone [33]. Although food consumption was not measured, 14-d-old pheasants fed Furadan-contaminated grain as their sole food source (carbofuran at 3.55 μg/g) grew at the same rate as control chicks [34]. Clay-colored sparrows fed grasshoppers containing carbofuran at 2.6 μg/g consumed more food than did those provided with unsprayed grasshoppers and showed no toxic effects or any loss in weight [35]. Although the pheasant chicks in our study that lost weight and died may have been those that were avoiding food, it is also probable that, as similar numbers of deaths and equal rates of weight loss occurred in the control groups, other factors were responsible. For example, dominant pheasant chicks may prevent inferior chicks from accessing the food dish, resulting in differential weight changes and survival [36].

The acute oral median lethal doses (LD50s) of chlorpyrifos, dimethoate, and carbofuran for 3-d-old pheasant chicks dosed using topical application to grasshopper carcasses are 25.8, 28.9, and 1.77 mg/kg body weight [P.A. Martin and D.L. Johnson, unpublished data]. Given a typical body weight of 30 g for 3-d-old pheasants, chicks in our study were consuming maximum doses equivalent to 1.3, 0.2, and 8.5 LD50 doses per day for the three insecticides, respectively (Table 6). The lack of toxic effects observed in our study may be due to the pattern of insecticide intake, distributed over approx. 14 h of daylight rather than as an acute dose. Carbofuran in particular is very quickly metabolized due to the rapid decarbamylation of AChE [37].

Depressed AChE activity of chicks exposed to contaminated food in the Furadan and Cygon treatments indicated that birds were receiving their primary intake of the insecticides in their diet rather than from the sprayed vegetation. Nevertheless, AChE activity was also reduced in chicks in the high-spray-rate Furadan treatment receiving uncontaminated food, although depression in this group was considerably less than that in the similar regime receiving contaminated food (23 vs. 36%). That there was significant depression in the unsprayed food treatment suggests that pheasant chicks were probably foraging for natural food in the sprayed grass and possibly receiving insecticides by preening contaminated feathers. Driver et al. [38] determined that preening and ingestion of contaminated food contributed equally to the depression of brain AChE activity in adult northern bobwhites exposed to simulated crop habitat sprayed with methyl parathion at 1.2 kg/ha in a wind tunnel. Quail in that study were present in the crop during spraying, however, which may have increased the amount of insecticide on the birds' feathers as well as uptake through inhalation. Dermal uptake through the feet is another method that may have contributed to the depressed AChE activity of pheasant chicks in our study [38]. That depression did not occur in chicks provided with unsprayed food at the lower Furadan rate or either of the two organophosphorus insecticide rates in our study suggests that uptake of insecticides via preening, foraging in sprayed grass, or dermal exposure is insufficient to affect chicks unless very high levels of a very potent cholinesterase inhibitor are used. These findings corroborate those of Martin et al. [7] and Martin and Forsyth [8] that mallard ducklings exposed to upland vegetation sprayed at one and two times the recommended grasshopper- control rates of Furadan had to consume contaminated food items from the vegetation in order to obtain sufficient insecticide to have a significant impact on cholinesterase and behavior.

Table 6.Pesticide intake by pheasant chicks feeding on grasshoppers contaminated with one of three insecticides

Insecticide	Nominal rate (g a.i./ha)	Insecticide concn. (μg/g)	Food consumed (g/chick-day)	Daily dose[a] (μg/g body wt)	Dose/LD50[b]
Lorsban	279	17.5	15.4	9.0	0.35
	1,116	78.0	13.0	33.4	1.29
Cygon	213	4.0	13.9	1.9	0.07
	852	14.9	12.2	6.1	0.21
Furadan	132	6.5	10.9	2.4	1.36
	528	36.6	12.4	15.1	8.53

[a]Calculated (food concentration [μg/g] × food consumed [g])/30 g, average body weight of 3-d-old pheasant chick.
[b]Based on values from Martin and Johnson (unpublished data) for 3-d-old pheasant chicks.

The lack of significant mortality of pheasant chicks in grassland plots freshly sprayed with insecticides at one and four times the rates of application recommended for grasshopper control suggests that the risk of acute toxicity presented by these chemicals to juvenile galliform species is fairly low. Behavioral effects apparent in the small number of chicks undergoing sublethal intoxication in our study could result in decreased survival of affected individuals in free-living broods. As well, the tendency toward lower weight gains in chicks in sprayed plots might be exacerbated in the wild by reductions in arthropod food resources throughout a brood's foraging range. Evidence from documented bird kills [4,5] suggests that adults of species that gorge themselves rapidly in sprayed areas, such as waterfowl and gulls, may be more vulnerable to acute insecticide poisoning compared to juvenile birds that consume much smaller quantities of contaminated food over a prolonged period of time.

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