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Northern Bobwhite Egg Matchability and Chick Immunocompetence Following a Field Application of Clopyralid

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Clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) is a phenoxy-picolinic, foliage-applied herbicide commonly used to manage honey mesquite *(Prosopis glandulosa)* and other brush species and broadleaf weeds on native rangeland (Valentine 1989). Broadcast application of herbicides to landscapes often occurs during the active nesting season of numerous bird species. A common herbicide recommendation is the broadcast application of 0.56 kg acid equivalent of clopyralid in 185 to 235 L of water per ha between 15 June and 15 August (Welch 1994). Broadcast application of clopyralid at this time coincides with the nesting period of Northern bobwhite *(Colinus virginianus)* (Lehmann 1984). Exposure of Northern bobwhite eggs to clopyralid is of concern, because some brush control agents are toxic to avian embryos. Though fosamine ammonium, a foliage applied herbicide, was reported to be relatively nontoxic with an LD_{50} of approximately 5,000 mg/kg for Northern bobwhite (Vallentine 1989), immersion of eggs in a 30% solution caused 95 to 100% embryo mortality (Hoffman 1983). Immersion of Northern bobwhite eggs in a 1.5% fosamine ammonium solution reduced chick body weight compared to controls (Hoffman 1983). Northern bobwhite egg viability and hatchability and growth and immune function after clopyralid exposure is uncertain.

Research approaches which involve immersing or injecting eggs in the laboratory with pesticide solutions and documenting subsequent malformations, growth depression, and mortality (Hoffman and Eastin 1981; Meneely and Wyttenbach 1989) do not closely mimic exposure of eggs to pesticides during actual field application. Further, impacts of sublethal exposure of vertebrates to pesticides can be less obvious than malformations and growth depression. Prenatal exposure of mammals to pesticides can result in significant decreases in immunocompetence (Barnett et al. 1980). Decreased immunocompetence may not influence survival in a pathogen-free laboratory environment, but may reduce

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survival in field environments. Unfortunately, few pesticides have been evaluated for immunotoxicity (Pruett 1992). The objective of this study was to determine if field application of clopyralid at the recommended label rate would alter viability and matchability of eggs and subsequently, growth and immune function of 28 day-old Northern bobwhite chicks.

MATERIALS AND METHODS

Northern bobwhite eggs were obtained from a hatchery (G.Q.F. Manufacturing, Savannah, GA) and randomly assigned to one of 36 experimental "nests." A "nest" consisted of six eggs held in a paper-mache tray designed for egg storage (G.Q.F. Manufacturing, Savannah, GA). All nests were placed in an insulated holding chamber and transported to the Range, Wildlife, and Fisheries Field Research Area in Lubbock, Texas.

The study area had a shrub stratum of honey mesquite and an herbaceous stratum of buffalograss *(Buchloe dactyloides)* and blue grama *(Bouteloua gracilis)* and was selected to allow passage of a 1.5-m boom sprayer. Vegetation in treatment areas (2 x 10 m) was reduced to a 2-cm stubble height and experimental nests placed systematically at 10 m intervals along the treatment area. Experimental design consisted of six replicates of two treatments: 0 and 0.56 kg a.e./ha of clopyralid. Clopyralid was mixed with water and applied with a 1.5-m boom sprayer mounted on a 4-wheeled all-terrain vehicle (ATV) equipped with an external 12-volt pump. Study areas were sprayed by centering the nests under the ATV to avoid crushing eggs. Nests treated with 0 kg a.e./ha of clopyralid were driven over with the sprayer apparatus disengaged. Average wind speed, temperature, and relative humidity during spraying were 9 km/hr, 31°C, and 37%, respectively.

Nests were removed from insulated chambers and placed on the ground, and eggs positioned on their longest axis immediately prior to spraying. We deliberately removed any vegetative canopy coverage above experimental nests prior to spraying. All treatment nests were completely sprayed in the process of spraying each area. It was our design to provide a worst-case scenario where eggs were afforded no foliage protection from spray. Nests were removed from each treatment area 15 min after exposure to clopyralid spray and returned to the insulated chamber. In the laboratory, eggs were removed from nests and incubated in plastic trays as recommended by Skewes and Wilson (1990) until hatch. The exterior of all eggs had dried before they were placed in the incubator. Upon hatching, chicks were weighed, banded, and placed in a brooder as described by Wilson and Dugan (1987). Chicks were weighed again at 7, 14, 21, and 27 days-of-age. Egg viability, egg hatchability, and Type I deformities as described by Kites and Suntornwat (1992) were recorded for each replicate.

Humoral and cell-mediated immunity of chicks was evaluated using the following protocol. Primary antibody response of chicks to sheep red blood cells (SRBC) was initiated by intramuscularly injecting chicks in the right pectorals at 22 days-of-age with 0.5 ml of a 10% SRBC suspension in phosphate-buffered saline (PBS) (Munns and Lament 1991). A Phytohemagglutinin-P (PHA-P) injection assay was used to evaluate T-cell mediated immunity. When chicks were 27 days-old they were injected intradermally in the right wing-web with 0.5 mg PHA-P in 0.1 ml of PBS (Lochmiller et al. 1993). The opposite wing-web (control) was injected with 0.1 ml of PBS. The thickness of each wing-web was measured at the injection site with a pressure-sensitive dial gauge just prior to and 24 hr after challenge. Cell-mediated immune response (wing-web index) was calculated as the difference in wing-web thickness between the PHA-P and PBS injected sites. Following wing-web measurement, chicks were anesthetized with 5 mg ketamine hydrochloride. Anesthetized chicks were exsanguinated via the jugular vein to obtain serum. Serum was stored at -85°C until analyzed. Serum anti-SRBC antibody titers were measured using a microhemagglutination assay and expressed as the reciprocal of the dilution (Wegmann and Smithies 1966). Differences in percent egg viability and hatchability, chick deformity, body weight, PHA-P wing-web response, and anti-SRBC antibody titer between treatments were tested using separate variance t-tests (n = 12 experimental units; Norusis 1990).

RESULTS AND DISCUSSION

We observed no ($P > 0.8$) differences between treatment groups with respect to percent egg viability and hatchability (Table 1), as both were within normal ranges (Schom and Abbott 1974). In early embryonic

Table 1. Effects of field-sprayed clopyralid exposure on percent egg viability and matchability, PHA-P wing-web responses, and anti-SRBC antibody titers (means ± SEM; n = 36 nests) of Northern bobwhite.

Clopyralid treatment	Percent viability	Percent matchability	Wing-web response	Anti-SRBC titer
0 kg a.e./ha	85.0 ± 3.51	65.1 ± 7.32	55.78 ± 2.05	2.63 ± 0.28
0.56 kg a.e./ha	83.9 ± 3.37	65.5 ± 4.27	51.55 ± 1.94	2.75 ± 0.31

development the most obvious pesticide-induced abnormalities are slower development, folding or undulation of the notochord and neural tube, and

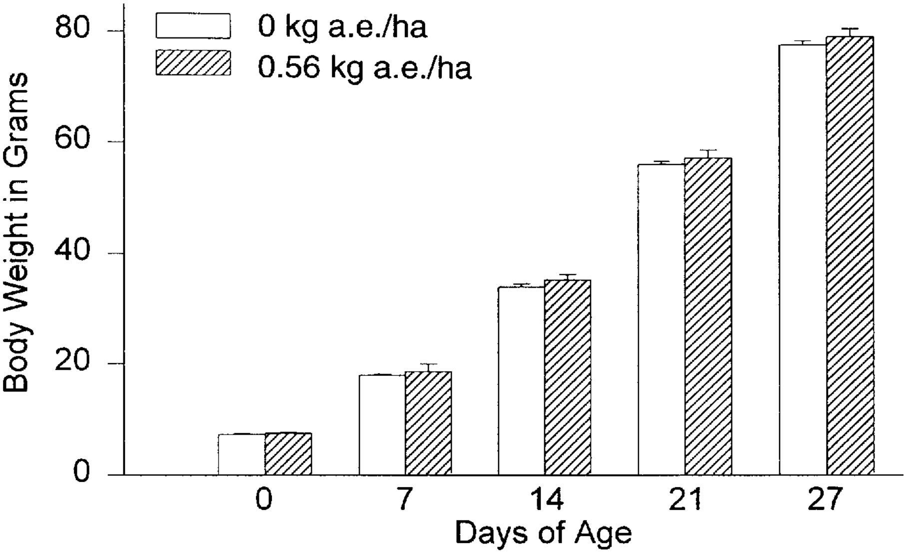

Figure 1. **Weight (mean ± SEM; n = 36 nests) of Northern bobwhite chicks at hatch, and 7, 14, 21, and 27 days of age after *in ovo* exposure to either 0 or 0.56 kg a.e./ha of clopyralid during field application.**

unilateral retardation of the cranial sense organs (Meneely and Wyttenbach 1989). No visible Type I malformations were evident in chicks in this study. Likewise, we detected no decrease in body weight (P > 0.27; Figure 1) or cell-mediated and humoral immunity (P > 0.16) of clopyralid-exposed chicks (Table 1). Exposure of birds to immuno-suppressive drugs or immunotoxins is known to decrease both the PHA-P wing-web and anti-SRBC antibody production responses (Rocke et al. 1984; Schrank et al. 1990). PHA-P wing-web indices in our study correspond to values previously reported for 7-week old Northern bobwhite chicks (Lochmiller et al, 1993). Anti-SRBC responses of Northern bobwhite in our study are lower than reported for 7-week old Northern bobwhite (Lochmiller et al. 1993). Lower anti-SRBC titers in our study are likely caused by differences in age (Munns and Lament 1991) and injection route. We conclude that clopyralid treatment did not impact cell-mediated and humoral immune system development relevant to recognition of and response to PHA-P and SRBC. Body weight of non-treated and clopyralid treated chicks in our study were normal (Serafin 1982; Dabbert et al. 1996). It appears that clopyralid dissolved in a water vehicle at the manufacturer-recommended concentration posed no detrimental effect to Northern bobwhite embryos when exposed during field application. Thus clopyralid can be used to manage woody brush cover in rangelands during Northern bobwhite egg laying and incubation periods with low probability of impacting embryo development and subsequent chick growth.

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