Effect of External Application of Pesticides to the Fertile Egg on Hatching Success and Early Chick Performance 1. Pre-Incubation Spraying with DDT and Commercial Mixtures of 2, 4-D: Picloram and 2, 4-D : 2,4,5-T

by
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LUTZ and LUTZ-OSTERTAG (1970)using avian eggs communicated that 2,4-D was not only embryocidal but teterogenic when externally applied. Following a spray treatment of 1 to 2 kg/ha on eggs under simulated field conditions, they reported mortality rates of 77, 43 and 77% for the pheasant (Phasianus colchicus L.), red partridge (Alectoris rufa L.)and grey partridge (Perdrix perdrix L.), respectively. Of those embryos surviving by the 19th day, there was a partial or total paralysis noted along with such anomalies as lordosis, fusion of neck vertebrae, muscular atrophy of legs, abnormal leg position, curled claws, and depigmented feathers. DUNACHIE and FLETCHER (1967, 1970) did not witness such physical changes when doses of 10-200 ppm 2,4-D were injected prior to incubation. However, hatchability was reduced up to 50%.

Mindful of the implications these results have on such common practices as roadside and aerial spraying, a study was initiated to re-investigate the effect that some common pesticides would have on avian development when externally applied. Residues were monitored to assess the degree of penetration into the egg.

Materials and Methods

The eggs used were derived from a commercial strain of Single Comb White Leghorn Hens that were all maintained on the same high quality ration and in the same facility. To assure a high degree of fertility, pooled semen from a like breed of cockerels was artificially placed in the oviduct. Each day's collection was distributed across all treatments to avoid confounding of results by age of the egg. Application of pesticides was accomplished by a calibrated spraying apparatus which passed over the eggs as would occur in the field. Because speed, pressure, height, and nozzle type were controlled, the rate of coverage in kg/hectare was reasonably accurate and repeatable.

The pesticides investigated were: a25% emulsion concentrate of technical DDT[1] ; a 2,4-D + picloram mixture[2]

[1]Niagra Chemicals, Burlington, Ontario.
[2]Trade name Tordon 101 Mixture, Dow Chemical Co., Midland, Mich.

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containing 39.6% 2,4-D and 10.2% picloram as the triisopropanolamine salts; and, a 2,4-D + 2,4,5-T mixture[3] containing 36% 2,4-D and 36% 2,4,5-T as the iso-octyl esters. Water served as the diluent in all cases. Eggs which acted as controls were sprayed with a volume of water corresponding to 746 l/ha. The volume application rates for the final solutions of DDT, 2,4-D + picloram and 2,4-D + 2,4,5-T were 280, 93.3 and 746 l/ha, respectively.

After spraying the eggs were allowed to dry over night (23.9°C) prior to initiation of incubation. A forced air incubator set at 37.5°C and 61% relative humidity was used for 19 days with a 90° tray rotation every hour. Non-viable and early dead germs (EDG) were subsequently removed upon transfer to a hatcher (3 days at 37.0°C and 61% relative humidity). Late dead germs (LDG) and pips were separated after hatching and examined macroscopically for gross abnormalities.

Chicks that hatched successfully were transferred to electrically heated raised wire floor brooder batteries and all given the same starting ration along with water on an ad libitum basis. In the first experiment, chicks were randomly sampled from groups representing each treatment and reared for a subsequent 3 week period. With the second experiment all birds were sexed at hatching and reared for 4 weeks.

Samples of egg, embryo and chicks from the treatments involving the highest concentrations of DDT and 2,4-D:2,4,5-T (11.2 kg/ha) were saved for analysis. DDT residues were determined by the method outlined by LANGLOIS et al. (1964). A Varian Aerograph Model 204 gas chromatograph equipped with a 250 mc tritium source electron capture detector and relying on a 182.9 x 0.32 cm glass column packed with 4% SE-30 + 6% QF-1 on chromosorb W was used for quantitation. Operating temperatures were 195, 200 and 230°C for the column, detector and injector, respectively. Nitrogen was the carrier gas with a flow rate of 80 ml/minute.

The 2,4-D and 2,4,5-T residues were recovered from the samples by the extraction procedure of YIP (1971). Quantitative determinations were made on a Varian Aerograph Model 1200 gas chromatograph equipped with an electron capture detector and 182.9 x 0.32 cm glass column with 6% Carborwax 20M on 60-80 mesh Varoport 30. Operating temperatures for the column, detector and injector were 190, 240 and 235°C, respectively. Carrier gas was nitrogen with a flow rate of 50 ml/minute.

Results and Discussion

Statistical analysis of the data illustrated on Table 1 indicated that there were no adverse effects on hatching success by external pre-incubation application of either DDT

[3]Trade name Esterone 3-3E, Dow Chemical Co., Sarnia, Ontario (Lot #7RH19, May 1971).

or mixtures involving 2,4-D, picloram and 2,4,5-T. The distribution of EDG and LDG regardless of the pesticide or its spray concentration were comparable with that of the control treatment. There were no morphological or behavioral abnormalities noted with any of the hatched chicks. Malformations were found in dead embryos only and then there was no meaningful relationship between treatment and incidence. The array of these aberations was typical of those noted under normal circumstances.

TABLE 1

Hatching Success and Incidence of Malformed Embryos Following Egg Pre-Incubation Pesticide Spraying[a]

Spray Treatment	Level (kg/ha)	% of Fertile Eggs EDG	LDG	Pip	Hatch	Malformed Embryos, %[b]
H_2O Control	-	6.1	3.1	3.0	87.9	33.3
DDT	1.1	6.3	1.6	1.1	90.7	37.6
	11.2	8.1	3.5	1.2	87.3	34.2
2,4-D + Picloram(4:1)	0.28	6.7	3.8	1.9	87.8	42.9
	2.8	7.8	2.0	1.8	88.3	34.5
2,4-D + 2,4,5-T(1:1)	1.1	7.6	3.4	1.6	87.5	23.7
	11.2	7.7	3.3	1.7	87.4	39.5

[a] 400 eggs set per treatment for each of 2 separate experiments. All data are the average of both trials.

[b] Expressed as a % of LDG and pip eggs.

The post hatch performance of the chicks from the first experiment was found to be significantly reduced by pesticide treatment ($P<0.05$), however, the failure to separate sexes seriously detracted from its credability. Rectifying this design flaw in the second experiment resulted in eliminating this negative effect and revealed a sex x treatment interaction ($P<0.075$). Treating the eggs with pesticides improved the growth of males but was without apparent effect on females. Further analysis indicated this improvement was restricted to those groups which had been treated with the lower level of DDT and the 2,4-D: 2,4,5-T mixture. Total mortality of the 2 experiments tended to be lower with all groups treated with pesticides relative to the control.

TABLE 2

Live Performance of Chicks from Eggs Sprayed with Pesticides Prior to Incubation

Spray Treatment	Level kg/ha	Expt. 1 (3 wk wt.,g)[a]	Expt. 2 (4 wk wt.,g)[a] ♂	Expt. 2 (4 wk wt.,g)[a] ♀	% Mort.
H_2O Control	-	181	234	198	8.6
DDT	1.1	170	257	199	4.3
	11.2	170	227	200	1.4
2,4-D + picloram(4:1)	0.28	168	233	209	2.8
	2.8	173	234	206	5.7
2,4-D + 2,4,5-T(1:1)	1.1	177	247	209	2.8
	11.2	181	258	208	4.3

[a]Each value is represented by 3 replicate groups of 10 chicks/pen.

Residues analysis of tissues from treatments involving DDT are shown on Table 3. The large quantities found on the shell substantiate the effectiveness of spraying as a contamination vector while the variety of residues involved reflects the technical grade of this insecticide. The amounts found on the outside of the egg versus that detected in the EDG indicates that little had penetrated the shell up to the time of death (3-5 days).

The considerably larger concentration of residues in the LDG suggests that pesticide had passed into the egg during the subsequent 14-16 day period of embryonic life. The predominance of DDE would support the contention of ECOBICHON and SASCHENBRECKER (1968) that this residue is the avian storage form of p,p′-DDT. Pipping through the shell immediately contaminates the embryo with the outside surface. Both pips and chicks had much greater concentrations of residues than the LDG.

Analyses for the phenoxyacetic acids from that treatment involving spraying of the 2,4-D + 2,4,5-T mixture also are shown on Table 3. Detection of both compounds in the dead germs indicates that these herbicides can also transverse the shell. However, the amounts found relative to concentration on the surface of the shell were much smaller than that noted with DDT and decreased with time.

TABLE 3

Residues Resulting from Pre-Incubation Spraying of Fertile Eggs with DDT or a Mixture of 2,4-D + 2,4,5 T (1:1)[a]

Tissues	Kg/ha Applied	DDT Residues, ppm of wet tissue					P.A. Residues, ppm wet wt[b]		
		DDE	p,p'-DDT	DDD	p,p'-DDT	Total	2,4-D	2,4,5-T	Total
Shell	0	-	-	-	-	-	-	-	-
	11.2	0.33±0.03	1.70±0.18	-	10.27±0.39	12.30±0.60	43.6	49.8	93.4
EDG	0	0.50	-	-	0.02	0.52	T	T	T
	11.2	0.52±0.03	T	-	T	0.52±0.03	0.55±0.11	0.41±0.10	0.96±0.21
LDG	0	0.32	T	0.05	0.06	0.43	T	T	T
	11.2	1.02±0.02	0.07±0.03	0.10±0.02	0.36±0.04	1.54±0.72	0.09±0.01	0.71±0.02	0.71±0.02
Pip	0	0.35	-	0.08	0.05	0.58	-	-	-
	11.2	2.37±0.03	0.34±0.07	0.34±0.7	2.64±0.65	5.69±1.41			
Chick[c]	0	0.42	0.03	0.08	0.15	0.65	T	T	T
	11.2	1.20±0.0	0.10±0.00	0.16±0.00	1.41±0.0	2.87±0.0	0.05±0.01	0.10±0.02	0.10±0.02

[a] Duplicate analysis have an associated standard deviation single analysis do not.
[b] % recovery of 2,4-D and 2,4,5-T were 55 and 65%, respectively.
[c] Chicks at hatch.

While the present experiments offer a reasonable parallel to what could occur by conventional field application of common pesticides, there are number of serious inadequacies. The first of these is that the egg from the domestic hen may not accurately reflect the effect on other species. Secondly, spraying over large areas usually encompasses eggs in the full spectrum of development. Eggs in the present study were treated while they were in a state of metabolic abeyance. On the other hand, LUTZ and LUTZ-OSTERTAG (1970) sprayed only 2,4-D and after several days development. Three to 5 days is known to be a particularly sensitive age in ovo. Furthermore, pesticide combinations may act different than if applied singly.

Summary

In 2 separate experiments, solutions of DDT, 2,4-D + picloram and 2,4-D + 2,4,5-T were sprayed on fertile chicken eggs preceeding incubation. No treatments were found to cause any adverse effect on hatching success, incidence of malformed embryos or subsequent chick mortality relative to control groups. Weight gain of male chicks from eggs treated with DDT (1.1 kg/ha) and the 2,4-D + 2,4,5-T combination (1.1 and 11.2 kg/ha) was found to be greater than that of control chicks. No changes were noted with the females. Residue analysis verified pesticide penetration into the egg.

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