Effect of External Application of Pesticides to the Fertile Egg on Hatching Success and Early Chick Performance 3. Consequences of Combining 2,4-D with Picloram and Extremes in Contamination.

by

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On the North American Continent use of 2,4-D in the control of broad leaf vegetation and its combination with picloram to further restrain nuisance brush is common. Application of both type herbicides is not restricted to farm-based operations but also involves large areas concerned with reforestation and rights-of-way maintenance. Under these circumstances, contact with several levels of animal life both domestic and wild is inevitable.

Although the data of PALMER and RADELELL (1969) on expected maximum exposure and toxicity of 2,4-D indicate a nil hazard for cattle, sheep and chickens, the report of LUTZ and LUTZ-OSTERTAG (1970) documents severe consequences on game bird eggs. Not only was extensive embryonic mortality observed but terata and anomalies were numerous. In contrast, SOMERS et al. (1973a) failed to encounter any adverse effects on the hen's egg by spraying 2,4-D in conjunction with picloram or 2,4-5-T prior to incubation. Responding to possible species differences, SOMERS et al. (1973b) subsequently performed a technically parallel study using a game bird previously reported to be vulnerable. As was witnessed with Gallus domesticus, eggs from the ring-necked pheasant (Phasianus colchius sp.) also did not adversely respond to contamination with these herbicides.

In further examining reasons for the aforementioned observed differences in effect which resulted from 2,4-D contamination, the present study considers the significance of herbicide combinations and extreme rates of application.

Materials and Methods

Two experiments separated by a 2 month interval were performed using fertile hens' eggs. All birds were of a commercial strain Single Comb White Leghorn and maintained in individual cages of the same facility. To assure a high degree fertility, each bird was artificially inseminated weekly with pooled semen from a like breed of cockerel. Both procedure and equipment involved in egg storage and incubation as well as herbicide application and analysis were identical to that previously described by SOMERS et al. (1973a).

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All herbicides used were commercially obtained. The 2,4-D: picloram (4:1) mixture[1] was certified to contain 39.6% 2,4-D and 10.2% picloram as the triisopropanolamine salts while the 2,4-D source[2] alone was in the amine form (600 g/l). Eggs representing the control were sprayed 24 hours prior to incubation with the equivalent volume of water that would be encountered by covering a hectare with 746 l. All treatments represented appropriate dilutions of herbicide to this relationship of liquid volume to contact area.

Designed to investigate the effect of combining herbicides, the first experiment involved using the 2,4-D:picloram mixture at 10x the suggested amount while 2,4-D by itself was applied at recommended dilution and 10x this value. In the second experiment, 2,4-D by itself was applied in graded quantities from the equivalent of normal to an extreme of 20 x recommendation. Vent sexing was performed on all chicks that successfully hatched. Random samples representing each treatment were reared for a subsequent 4 weeks in pens of electrically heated raised wire floor brooder batteries. All birds received the same starting ration along with water ad libitum.

Results and Discussion

Regardless of whether 2,4-D was sprayed on the fertile egg by itself or in combination with picloram, contamination did not lead to any adverse effects on incubation performance (Table 1). Increasing the rate of 2,4-D application progressively from normal to 20x expected usage in a second experiment was also without effect. Overall hatching success in both cases was in the realm of expectation and comparable. The considerably larger proportion of malformations encountered with the late dead germs and pipped eggs by the first over the second test can be attributed to subtle differences in incubator operating conditions.

There was also no indication of a reduced performance after hatching because of herbicide contact with the egg (Table 2). On the contrary, gain was improved from 3 to 4 weeks of age at a time when growth rate is maximized and any contaminants concentrated in the yolk sac would have been resorbed into the system. A statistical partitioning of this effect in the first experiment showed the increased gain to be restricted to the males which had been subjected to either of the 2 levels of 2,4-D alone but not when the

[1] "Tordon 101", Dow Chemical Co., Midland, Michigan. Lot #595357.
[2] "2,4-D Amine 80", Dow Chemical Co., Midland, Michigan. Lot #FC-708.

TABLE 1

Incubation Performance of Hens' Eggs Sprayed With Commercially Available Herbicide Formulations

Application Herbicide	kg/ha	% of Viable Germs[a] EDG	LDG	Pip	Hatch	% of (LDG + Pip) Malformed
EXPERIMENT I[b] (Ingredient Separation of Multiple Formulation)						
Water Control	-	8.1	10.8	4.5	73.3	23.0
2,4-D	3.4	7.8	6.9	2.4	82.8	21.4
	33.6	10.4	4.5	4.4	80.8	26.3
2,4-D:Picloram	28.0	9.3	6.4	5.3	78.7	24.1
$S\bar{x}$ (2df)	-	6.8	5.7	6.0	6.8	-
EXPERIMENT II[c] (Extreme 2,4-D Field Application Rates)						
Water Control	-	10.2	6.3	4.2	78.9	12.5
2,4-D	2.8	7.3	4.7	2.1	85.0	11.5
	5.6	8.4	5.0	2.9	83.1	10.0
	11.2	9.9	4.3	0.8	85.0	10.5
	22.4	8.8	5.6	3.2	80.9	9.1
	44.8	7.5	6.0	5.2	80.0	16.6
$S\bar{x}$ (5df)	-	9.5	9.6	7.9	7.7	-

[a] Early dead germs (EDG) refer to all mortality *in ovo* prior to 18 days incubation while late dead germs (LDG) are subsequent failures that did not pip the shell.

[b] Data relative to the control represents one group of 350 eggs while 2 such replicates form the values of each herbicide treatment.

[c] All data is represented by 400 eggs set/treatment.

combination with picloram was employed. However, no live performance advantages were discernable in the second experiment regardless of the 2,4-D application rate. Treatment effects on feed utilization and mortality were not readily apparent with either trial.

Analyses to assess the degree of contamination were only performed on samples from Experiment II where 2,4-D spray concentration was the variable (Table 3). Herbicide recovery from the shell surface paralleled increases expected from the progressively greater application rates employed. Examination of the contents from the unincubated egg showed that a comparable response was not occurring internally. Even though external 2,4-D amounts went through a 20 fold increase, only a tripling of concentration occurred in the albumen plus yolk.

After incubation, dead germs were found to contain decreasing quantities of 2,4-D with embryonic age which maintained a corresponding relationship to initial degree of contamination. Thus, biological activity in conjunction with incubation environment did not assist undue amounts of this phenoxyacetic acid to enter the egg after wet contact. Contrary to expectation, the body of the late dead germ concentrated more of this lipophilic categorized compound than was found with the high fat contents of its associated yolk sac. No doubt, the larger and variable amounts found with the chick resulted from shell surface contact at hatch.

For all practical purposes, the present study which examined the consequences of combining 2,4-D with picloram and subjected the egg to extremes in contamination failed to cause any adverse effects on either hatching success or early chick performance. In accord with earlier reports (SOMERS et al. 1973 a,b), the only measurement which indicated any response to herbicides was an improved body weight gain. Though encounter

TABLE 2

Live Performance of Chicks from Hens' Eggs Sprayed With Commercially Available Herbicide Formulations[a]

Application		♂(3-4 weeks)		♀(3-4 weeks)		% Tot.
Herbicide	kg/ha	Gain,g	F/G[b]	Gain,g	F/G	Mortality
EXPERIMENT I - (Ingredient Separation on Multiple Formulation)						
Water Control	-	78	2.28	69	2.38	2.0
2,4-D	3.4	90	2.17	65	2.45	1.0
	33.6	88	2.19	73	2.35	0.0
2,4-D:Picloram	28.0	82	2.29	72	2.36	2.0
$S\bar{x}$ (df)	-	16(106)	0.12(6)	20(108)	0.21(6)	-
P <	-	0.007	-	0.254	-	-
EXPERIMENT II - (Extreme 2,4-D Application Rates)						
Water Control	-	79	2.30	-	-	2.7
2,4-D	2.8	84	2.36	-	-	8.7
	5.6	76	2.34	-	-	2.7
	11.2	81	2.20	-	-	2.7
	22.4	79	2.26	-	-	0.0
	44.8	82	2.39	-	-	2.7
$S\bar{x}$ (df)	-	24(161)	0.13(10)	-	-	-
P <	-	0.855	-	-	-	-

[a] All data in each Experiment is represented by 3 replicate groups of 12 chicks/sex for each treatment for the interval from 3 to 4 weeks of age.

[b] Feed Consumed/body weight gain.

TABLE 3

Recovery of 2,4-D From Hens' Eggs After Extreme Spray Application
(ppm wet tissue)[a]

Application		Pre-Incubation		Post Incubation			
Herbicide	kg/ha	Shell	Interior	EDG	LDG	LDG Yolk	Chick
Control	-	-	T	0.04	T	T	0.02
2,4-D	2.8	26.6 ± -	0.49 ± 0.06	- ±	-	-	0.31 ±
	5.6	55.7 ± 10.5	-	0.30 ± 0.10	- ±	- ±	0.31 ± 0.19
	11.2	117.6 ± 7.9	0.51 ± 0.09	- ±	0.46 ± 0.19	0.22 ± 0.06	1.31 ± 0.33
	22.4	161.9 ± 46.1	0.71 ± 0.12	0.59 ± 0.19	- ±	- ±	2.80
	44.8	439.6 - 10.9	1.34 ± 0.05	1.72 ± 0.02	0.81 - 0.30	0.52 ± 0.04	10.76 ± 0.45

[a] ± Duplicate analysis have an associated standard deviation, single analysis do not. The % recovery of 2,4-D was 55% as based on the extraction procedure of YIP (1971) and electron capture GLC using 6% carbowax 20 M on 60-80 Mesh Varoport 30.

in this respect was variable, the consistent observation that only the male was ever affected is pertinent considering the alterations in sex hormones caused by similarly structured compounds.

Using a post-mitochondria hepatic fraction in conjunction with a NADH generating system, NOWICKI and NORMAN (1972) reported that the male 2 week old chick metabolized nearly twice as much testosterone, 4-androstene-3,17-dione and estadiol-17β as the female. By orally preconditioning these birds for 7 days with 1 mg/day of o,p'-DDT, p,p'-DDT, or PCB's (Aroclor 1254 or 1260) values for both were increased approximately 3 fold. WHITEHEAD and PETTIGREW (1972) made note of numerically greater gains made by unsexed day old chicks dosed with 10 and 100 mg 2,4-D/kg of body weight/day for 4 weeks. Although these weight improvements have not been considered "adverse" hormonal alterations as the possible mode of herbicide action point to a need for knowing long-term effects, particularly on reproduction.

Summary

Aqueous solutions of 2,4-D at recommended and 10x field rates, along with its combination with picloram (4:1) at the higher level were sprayed on hens' eggs prior to incubation. No adverse effects resulted with respect to either incubation or subsequent live performance of hatched chicks. A follow-up experiment using graded levels of 2,4-D to an extreme of 20x average application also failed to provoke any pre- or post-embryonic problems. Residue analyses verified proportional external contamination of the egg with spray concentration, however, examination of the internal contents showed entry to be far less than proportionate. Biological activity and/or incubation did not assist further *in ovo* contamination after initial wet contact.

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