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Hatching Success and Early Performance of Chicks from Eggs Sprayed with 2,4-D, 2,4,5-T and Picloram at Various Stages of Embryonic Development[1]

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LUTZ-OSTERTAG and LUTZ (1970) reported that topical applications of 2,4-D to game bird eggs would seriously disrupt normal embryogenesis and reduce hatching success. SOMERS et al. (1974 c) did not observe any alterations in hatchability, incidence of terata or early chick performance following external applications of 2,4-D or 2,4-5-T both in combination with picloram to hens' eggs at normal and 10x advocated field concentrations. Similarly treated Ringed Neck Pheasant eggs in a subsequent study yielded comparable results thereby reducing the likelihood of species differences for the absence of adverse effect. Parallel efforts which removed the possibility of a synergism due to the phenoxyacetic acid:picloram combinations and employed exaggerated levels of 2,4-D alone also failed to elicite a differential response when compared to appropriate controls (SOMERS et al., 1974 b). In agreement, GROLLEAU et al. (1974) also failed to observe any effects of contaminating partridge and quail eggs with 2,4-D.

It is particularly important to note that both SOMERS et al. (1974 a,b,c) and GROLLEAU et al. (1974) limited egg treatment to that period prior to incubation when the germ was in a state of metabolic abeyance. Realistically, eggs as they occur in the wild would be in a wide developmental array when contaminated. The presence study examined the significance of embryological age on the hazard of herbicide encounter.

Materials and Methods

Chick embryonic mortality is normally concentrated at

[1] The data contain herein were excerpted from a Ph.D. thesis submitted to the University of Guelph (1975) by the senior author (J.D.S.).

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3 to 5 and 18 to 20 days of development. These stress periods were selected as the most crucial times of contamination with herbicide application being specifically at 0, 4 and 18 days of incubation. In addition treatment with 2,4-D, 2,4-5-T and picloram, 3 control groups were also involved for each embryonic age chosen for application to assess non-herbicidal influences. One control lot of eggs was transported to spraying facilities without further manipulation. A second group was transported and sprayed with water only. The third group consisted of an application of formulating ingredients that would be common to 2,4,5-T and picloram appropriately diluted to field circumstance but exclusive of the active components. One additional lot of eggs was set without any handling or treatment to serve as the incubator control.

Twenty-four successive days' eggs were obtained from a commercial strain of Single Comb White Leghorn hens. To assure fertility all birds were inseminated twice, 3 days apart, initially before collection started then once every week for 4 weeks during collection. All semen was pooled from a like breed of cockerels to further enhance the likelihood of fertilization. The hens were maintained individually caged in the same house and fed the same ration. Eggs were collected daily with an equal number being used from each 2 days' successive collection to form 12 groups representing days of storage prior to incubation across each of the 18 treatment combinations and incubator control (8,208 eggs in total).

The facilities involved in egg storage, incubation and herbicide application, as well as the specifics in their operation, have been described previously (SOMERS et al., 1974 c). All herbicides along with the formulation blank were applied in the spray volume of 746 l/ha. Commercially available preparations of 2,4-D[1], 2,4-5-T[2] and picloram[3] were diluted in relation to this volume to supply 10x the normal amounts used in practice (11.2 kg/ha) with the blank[4] supplying corresponding quantities of non-active ingredients.

To assess the total number of viable germs all eggs failing activity were opened. Late dead germs (LDG), pipped dead germs (PDG) and all chicks were examined for gross abnormalities. Following vent sexing of normal appearing chicks, 2 replicate groups of males and females were saved from eggs originally stored prior to incubation for the extreme periods of 1-2 and 23-24 days. Live performance was monitored for 4 weeks in thermostatically controlled raised wire floor brooder batteries with ad libitum feeding of the same composition starting ration. All birds were subsequently pooled and transferred to floor pens of a rearing facility until the males were 20 and females 16 weeks of age. During this time body weights were measured and all mortality submitted to the Ontario Veterinary College for a post-mortem examination.

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[1] Trade name Esteron 99, Dow Chemical Co., Midland, MI.
[2] Trade name Esteron 245, Dow Chemical Co., Midland, MI.
[3] Trade name Tordon 22K, Dow Chemical Co., Midland, MI.
[4] Formulated and supplied by Dow Chemical Co., Midland, MI.

Results and Discussion

A statistical evaluation of all data associated with incubation failed to disclose a significant interaction involving spray treatment and the stage of embryonic development at application. Thus, at any point before or after incubation a difference in the consequences of herbicide contamination on the fertile egg is unlikely. The data on Table 1 is partitioned into the experiment's main effects. Paralleling the results of earlier studies

TABLE 1

Incubation Performance of Experimental Hens' Eggs[a]

Variable	% of Viable Germs[b] EDG	LDG	PDG	Hatch	Malformations % of VG-EDG[c]
Effect Due to Spraying and Herbicide Source					
Controls					
Incubator	7.9	7.2	9.6	71.7	1.8
Transportation	6.5	6.1	6.0	76.7	1.3
Water Spray	7.7	5.2	6.2	76.4	1.4
Blank Spray	7.7	4.4	5.5	78.0	1.7
Herbicides					
2,4-D	7.1	7.3	8.5	72.3	1.6
2,4-5-T	7.4	7.5	8.1	73.3	1.3
Picloram	8.1	6.1	8.0	72.4	1.0
Effect Due to Stage of Embryonic Development When Contaminated					
Incubation Age, Days					
0	7.2	6.5	9.3^{d}_{de}	73.2	1.6
4	7.1	6.0	6.9^{f}	74.6	1.4
18	8.1	5.9	5.1^{f}	76.5	1.3
σ (198 df)	5.9	7.3	7.5	6.1	0.8

[a] All means are based on 432 eggs over 12 replicates per treatment for each of 3 stages of embryonic development.

[b] Expressed as % of viable germs. Total viable germs being 91.6% with no significant effects due to spray treatment, embryonic stage of development and their interactions ($P > 0.05$). EDG= early dead germs (deaths prior to 6 days); LDG=late dead germs (deaths after 6 days without breaking shell); PDG=pipped dead germs (deaths after shell broken but failure to hatch).

[c] Expressed as a percentage of viable germs less the contribution from EDG.

[def] Pertains to Duncan's Multiple Range Test. Values in the same column with the same superscript letter are not significantly different ($P < 0.05$).

(SOMERS et al., 1974 abc; GROLLEAU et al., 1974), spraying and herbicide source had no adverse influence on any parameter used to evaluate hatching success ($P < 0.05$). If one considers the eggs representing the incubator control as an optimal circumstance then from a consistent numerical standpoint, spraying with or without herbicides is an advantage.

Regardless of whether the egg was unincubated or embryogenesis had ensued to either of the 2 stages of development employed there was little influence on ultimate hatching success by any spray or herbicide treatment. An increase in egg moisture as the result of day 18 spraying may account for the reduction in pipped dead germs, however, this effect was advantageous. HILBIG et al. (1976) spraying Japanese quail eggs with 10x normal dosage of 2, 4-D or a 2,4-D: 2,4-5-T mixture prior to and after 3 days incubation also failed to note any adverse effects on hatching rate, body weight and malformations of offspring.

TABLE 2
Live Performance of Chicks From Experimental Hens' Eggs[a]

Variable	Weight gain, g[b] ♂	Weight gain, g[b] ♀	% Mortality ♂	% Mortality ♀
Effect Due to Spraying & Herbicide Source				
Control				
Incubator	238	208	0.0	0.0
Transportation	240	212	3.5	2.0
Water Spray	245	210	0.0	1.4
Blank Spray	242	215	0.0	3.9
Herbicides				
2,4-D	235	217	0.0	2.5
2,4-5-T	238	219	3.8	4.0
Picloram	237	219	3.3	4.0
Effect Due to Embryonic Development When Contaminated				
Incubation Age, Days				
0	239	215	0.7	2.3
4	241	218	1.9	4.7
18	240	211	2.8	2.1
σ(df)	35(467)	27(438)	1.4(17)	1.6(17)

[a] Means are based on 25 chicks/sex over 2 replicates per treatment for each of the 3 stages of embryonic development.

[b] The average chick hatching weights of males and females were 37.5 and 37.1 g, respectively. There were no statistically significant effects due to spray treatment, embryonic stage of development and their interactions ($P > 0.05$).

Not shown are the data relative to the effect of duration of egg storage prior to incubation. As is commonly observed, there were significant adverse effects for all parameters used to evaluate incubation as egg storage time increased ($P < 0.05$). There were no statistically significant interactions with either of the primary variables of spray treatment or stage of development at application. These results indicate that the germ as weakened by the consequences of prolonged storage prior to incubation, was not any more susceptible to the hazards of herbicide contamination or its associated procedures than the optimal circumstance involving the new laid egg.

Chicks from eggs stored 1-2 and 23-24 days prior to incubation did not give a differential response in terms of weight gain or mortality either when spray treatment or embryonic age upon contamination was varied (Table 2). Although eggs stored for a prolonged period of time do not hatch as well as when they are fresh, data not shown indicate that chicks from both sources perform comparably with no detectable interactions associated with the primary variables as shown on Table 2. Extending the live performance period to 16 weeks for the females and 20 weeks for the males did not result in any alteration of results encountered during the first 4 weeks.

Summary

Aqueous solutions of 2,4-D, 2,4-5-T and picloram were sprayed on hens' eggs at 10x normal rate (11.2 kg/ha). Eggs used were stored prior to incubation over a 24 day period and application on each age egg occurred at the start as well as 4 and 18 days after the onset of embryonic development. No adverse effects on any parameter used to evaluate either incubation or subsequent live performance could be attributed to either spray treatment or embryonic age when applied. Although extended preincubation storage significantly reduced hatching success, it was independent of herbicide contact and germ stage when contaminated. Chicks derived from eggs stored 1-2 and 23-24 days before incubation performed comparably and were uninfluenced by all treatments during incubation.

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