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Brain ChE Response in Forest Songbirds Exposed to Aerial Spraying of Aminocarb and Possible Influence of Application Methodology and Insecticide Formulation

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Development of a particular insecticide formulation for use in forest spraying in Canada normally follows a prescribed series of steps in order to furnish the information requirements of pesticide registration protocol. If the formulation is found to be effective and environmentally acceptable it may be registered for its intended operational use. During the early stages of insecticide formulation development certain changes in application methodology may occur. Eventually the operational application method may be quite different from any of the experimental ones even though the insecticide formulation and application and dosage rates remain unchanged.

During the many years of spraying against spruce budworm (Choristoneura fumiferana) in eastern Canadian forests there have been incidents suggesting that application methodology can influence the impact of a particular insecticide on songbirds. For example, PEARCE et al. (1979) showed that songbirds foraging in the upper canopy of forest sprayed with fenitrothion were significantly more affected in areas sprayed by TBM aircraft than in areas sprayed by DC-6 aircraft: although formulations differed the dosage rate was constant.

Recently, we had the opportunity of conducting studies of a new preparation of aminocarb (4-(dimethylamino)-3-methylphenol methylcarbamate) known as Matacil® 180 Flowable (180F). Initially we reported that there was no significant impact on songbirds when the spray was applied by Cessna 188 Ag-Truck (BUSBY et al. 1982). In the present paper we report the results of subsequent experimentation in the development of Matacil® 180F, in which application of the insecticide was by TBM aircraft. Two formulations were used: one was an emulsion identical to the one employed in the initial investigation and one was an oil-based formulation. Both were applied at the same dosage and application rates. Songbird response was determined by measuring brain cholinesterase (ChE) activity in specimens collected in sprayed and unsprayed forest.

MATERIALS AND METHODS

The study areas were located in New Brunswick within the Maritime Lowlands Ecoregion (LOUCKS 1960). The forest is characterized by red spruce (Picea rubens), black spruce (P. mariana) and balsam fir (Abies balsamea). Those species are associated with a smaller component of red maple (Acer rubrum), eastern white pine (Pinus strobus) and white birch (Betula papyrifera). The two spray blocks were scheduled for treatment in an ongoing annual spray program and were sprayed within the operational framework of activities - only the pesticide formulations applied were experimental. Technical details of the spray applications are given in TABLE 1.

TABLE 1. Technical detail of spray regimes and applications.

	BLOCK 82	BLOCK 86
Location	25 km NW Fredericton	30 km SW Fredericton
Size	5000 ha	3280 ha
Formulation (by volume)	25.93% Matacil® 180F 74.07% Insecticide Diluent 585 oil	25.93% Matacil® 180F, 1.27% Atlox 3409F®, 72.8% water
Dosage rate (active ingredient)	70 g/ha	70 g/ha
Application rate (total formulation)	1.46 ℓ/ha	1.46 ℓ/ha
Date and time of application:		
Spray 1	June 4, 06:30-07:05	May 31, 19:08-19:33
Spray 2	June 9, 05:50-06:24	June 8, 05:54-06:29
Aircraft type	team of two Grumman Avenger TBMs	
Spray equipment	boom and nozzle, T-Jet nozzles, 110-10 tips	
Aircraft guidance	aerial flagging system (Flieger 1964)	

Songbirds were collected (.410 shotgun) in each block within 48 h of spraying. Only adult males were taken. Although most birds were located by cueing into singing activity, we also attempted to collect non-singing males. Control birds were collected May 29 to May 31 from unsprayed forest adjacent to block 86. Birds in which the brain received heavy shot damage were not included in the analyses. The species were chosen to represent all foraging levels in the forest. Tennessee Warblers (Vermivora peregrina) and Bay-breasted Warblers (Dendroica castanea) are both fairly wide-ranging but primarily upper-strata foragers. Magnolia Warblers (D. magnolia) are found at lower levels, typically in young coniferous growth, and American Redstarts (Setophaga ruticilla) are essentially lower-to-middle-strata foragers which occur mainly in localized areas of deciduous regeneration and along stream banks. White-throated Sparrows (Zonotrichia albicollis) are ground-to-lower-canopy foragers.

After collection, the whole brain of each specimen was immediately placed in a serum vial and stored in liquid nitrogen (-196°C) until assayed, a maximum of 13 days later. Brain ChE activity was determined colorimetrically using the technique of ELLMAN et al. (1961) as modified by HILL and FLEMING (1982).

TABLE 2. Mean brain ChE activity (mU/mg brain) in forest songbirds: comparison of unsprayed samples and samples collected in blocks sprayed with aminocarb in emulsion (block 86) and oil (block 82).

Species	Unsprayed	Block	Sprayed: First application, 0-24	Sprayed: First application, 25-48	Sprayed: Second application, 0-24	Sprayed: Second application, 25-48
			Time period (h) post-spray when birds collected			
Tennessee Warbler	27.9|10|	86	24.8(-11.1)|7|†	25.9(-7.2)|6|	26.0(-6.8)|7|	27.0(-3.2)|6|
		82	28.8(+3.2)|10|	32.8(+17.6)|6|	-	28.5(+2.2)|6|
Bay-breasted Warbler	32.8|11|	86	27.8(-15.2)|6|	26.6(-18.9)|5|	30.9(-5.8)|9|	27.7(-15.6)|7|
		82	32.7(-0.3)|5|	33.0(+0.6)|7|	-	29.7(-9.5)|10|
Magnolia Warbler	30.9|11|	86	30.3(-1.9)|8|	28.0(-9.4)|4|	29.2(-5.5)|6|	29.6(-4.2)|7|
		82	30.5(-1.5)|7|	35.4(+14.6)|8|	-	28.2(-8.7)|7|
American Redstart	28.7|11|	86	25.3(-11.9)|5|	29.6(+3.1)|5|	28.6(-0.4)|5|	28.3(-1.4)|5|
		82	30.6(+6.6)|4|	-	-	-
White-throated Sparrow	30.9|11|	86	30.2(-2.3)|5|	31.0(+0.3)|5|	29.3(-5.2)|4|	30.5(-1.3)|6|
		82	31.0(+0.3)|5|	31.5(+1.9)|5|	-	30.3(-1.9)|6|

† Mean brain ChE activity (percent change from unsprayed birds)|n|. Underlined figures are significantly different from mean of unsprayed sample at 0.05 level of significance, Mann-Whitney U-test.

RESULTS

Most species-samples from the emulsion-sprayed block exhibited some brain ChE inhibition (TABLE 2), Tennessee, Bay-breasted and Magnolia Warblers being most affected. Statistically significant inhibition was found in 4 of those 20 samples, Bay-breasted Warblers showing the most consistent reduction. Birds collected from the oil-sprayed block were apparently less affected and some birds collected after the first spray application had substantially increased brain ChE activity. Brain ChE activity was similar in birds collected from both blocks after second sprays. Neither block contained any species-samples in which brain ChE inhibition was greater than 20% of that in controls. There were no obvious general relationships between time after spraying and brain ChE activity, except that after the first treatment of the oil-sprayed block there was an increase in brain ChE activity, particularly in Tennessee and Magnolia Warblers in the 25-48 h sampling period.

Different trends were apparent when brain ChE activities in birds taken after the first spray application were compared with those in birds taken after the second (TABLE 3). In the emulsion block, brain ChE activities generally increased after second application whereas in the oil block a general decrease was observed. There were no obvious species-specific ChE trends in either block.

TABLE 3. Mean brain ChE (mU/mg brain) in forest songbirds: comparison of activities in samples after first and second application of aminocarb in emulsion (block 86) and oil (block 82).

Species	Block	Spray application		Percent change
		First	Second	
Tennessee Warbler	86	25.3±5.5	26.5±2.1	+4.7
	82	30.3±4.6	28.5±1.3	-5.9
Bay-breasted Warbler	86	27.3±3.7	29.5±2.9	+8.1
	82	32.9±2.7	29.7±3.1	-9.7
Magnolia Warbler	86	29.5±3.2	29.4±5.0	-0.3
	82	33.0±5.8	28.2±4.2	-14.6
American Redstart	86	27.4±3.6	28.4±2.8	+3.7
	82	30.6±2.2	-	-
White-throated Sparrow	86	30.6±1.9	30.0±2.5	-2.0
	82	31.5±2.6	30.3±3.3	-3.8

The impact of sprays on individual birds is given in TABLE 4. In both blocks, Tennessee, Bay-breasted and Magnolia Warblers were somewhat more affected than the other species. There were no general trends suggesting increasing depression or recovery with time.

TABLE 4. Number of songbirds with brain ChE inhibited by more than 20% of controls: comparison after first and second applications of aminocarb in emulsion (block 86) and oil (block 82).

Species	Block	Spray application: First — Time period (h) post spray when birds collected: 0-24	First: 25-48	Second: 0-24	Second: 25-48	Total
Tennessee Warbler	86	2(29)†	1(17)	0(0)	0(0)	3(12)
	82	2(20)	0(0)	-	0(0)	2(9)
Bay-breasted Warbler	86	2(33)	1(20)	0(0)	1(4)	4(15)
	82	0(0)	0(0)	-	2(20)	2(9)
Magnolia Warbler	86	0(0)	1(25)	1(17)	1(14)	3(12)
	82	2(25)	0(0)	-	2(29)	4(18)
American Redstart	86	0(0)	0(0)	1(25)	0(0)	1(5)
	82	0(0)	0(0)	-	0(0)	0(0)
White-throated Sparrow	86	0(0)	0(0)	0(0)	0(0)	0(0)
	82	0(0)	-	-	-	0(0)
Total	86	4(13)	3(12)	2(6)	2(6)	11(9)
	82	4(13)	0(0)	-	4(14)	8(9)

† Number in parentheses is percent of sample.

DISCUSSION

Our study indicates that in the New Brunswick forest spray context application of an emulsion of Matacil® 180F induces measurable depression of brain ChE activity in forest songbirds. In an earlier study (BUSBY et al. 1982) of an experimental spray of the same formulation by a rotary atomizer-equipped Cessna 188 Ag-Truck we were unable to detect significant brain ChE depression in songbirds. We suggest, therefore, that the manner of spray delivery and emission can influence the degree of impact on birds.

The lack of depression of brain ChE activity in birds from the oil-sprayed block indicates that the manner of insecticide formulation may also be an important variable. That could not be verified, however: an aberrant spray application may have resulted in some of the birds being collected from unsprayed sections of the block. The substantial increase of brain ChE activity in some samples from the oil-sprayed block cannot be explained. It is unlikely that laboratory procedure was the cause as samples were selected randomly during assays. All field sampling was accomplished within a two-week period so possible seasonal variability of brain ChE activity (MITCHELL and WHITE 1982) is an unlikely factor. We are not aware of any other reports of increases in brain ChE activity in birds exposed to ChE-inhibiting pesticides.

The evidence that upper-canopy foragers are more affected than low-level ones is consistent with conclusions drawn in similar studies in which impacts were observed (e.g. MOULDING 1976, ZINKL et al. 1977, PEARCE et al. 1979). The lack of effect on White-throated Sparrows in the present study contrasts with the finding elsewhere (BUSBY et al. 1981) that that species was affected as much as upper-canopy foragers. An explanation may lie in the fact that in the earlier investigation White-throated Sparrows were collected from forest clear-cuts whereas in the present one they were taken in closed-canopy forest. This supports our contention that birds in open-canopy habitat are relatively more vulnerable to aerial spraying of pesticides.

It is unclear what aspects of spray application methodology are most relevant to impact on songbirds. Some variables are concomitant with others, e.g. a change of aircraft type will result in different air speed, altitude and maneuverability in adjusting to uneven terrain while spraying. Changes in spray hardware (e.g. rotary atomizer vs. boom and nozzle) and morning vs. evening spraying may also be relevant to environmental impact (BUSBY et al. 1981). Differences in weather at times of spray applications compound the difficulty of identifying the most pertinent factors.

We conclude that a spray impact was detected but that it was a minor one. Application of the criteria of LUDKE et al. (1975) - 20% brain ChE inhibition in birds indicates exposure to the pesticide and 50% inhibition suggests some mortality - reveals the likelihood that in our study no birds were killed as a result of spraying and that sub-lethal effects were few. Although we observed no individual birds exhibiting symptoms of carbamate poisoning, twice after spraying (once in each block) birds were noted to be unusually quiet and restless, making collection very difficult. Those observations and the brain ChE analyses support our belief that spraying by TBM aircraft with boom and nozzle equipment under operational spraying conditions is likely to have a slightly greater impact on songbirds than spraying by Cessna aircraft fitted with rotary atomizers and operating under experimental conditions. The factors accounting for that difference remain unidentified.

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