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Reproduction and nest behaviour of Tennessee warblers *Vermivora peregrina* in forests treated with Lepidoptera-specific insecticides

STEPHEN B. HOLMES

Natural Resources Canada, Canadian Forest Service, 1219 Queen St. East, PO Box 490, Sault Ste Marie, Ontario, Canada P6A 5M7

Summary

1. This study was designed to test the hypothesis that food reductions caused by forest spraying with Lepidoptera-specific insecticides would affect Tennessee warbler behaviour and reproduction.

2. Tennessee warbler nests and parental behaviour were monitored in two spray blocks, one treated with *Bacillus thuringiensis* (*Bt*) and the other with tebufenozide (MIMIC), and in an untreated control area.

3. Nestling survival and growth were unaffected by the insecticide treatments. Nests in the treated blocks had smaller clutches, smaller broods and lower hatch rates than nests in the control block, but these differences were not statistically significant.

4. Nestling diets were similar in the MIMIC and control blocks.

5. There were slight differences in the behaviour patterns of female Tennessee warblers in the MIMIC and control blocks. MIMIC females spent less time at the nest and more time foraging.

6. The results of this study suggest that the indirect effects of forest spraying with Lepidoptera-specific insecticides pose little risk to forest songbirds.

Key-words: Bacillus thuringiensis, indirect effects, insectivorous forest songbird, MIMIC, tebufenozide.

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Introduction

Forest spraying to control spruce budworm *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) has been an annual event in eastern Canada since the early 1950s. During most of these years, broad spectrum chemical insecticides were used (Prebble 1975; Armstrong & Ives 1995). More recently, however, an attempt has been made to reduce the non-target impacts of forest spray operations by using more selective insecticides (Ennis & Caldwell 1991).

Microbial insecticides based on the bacterium *B.t.k.* (*Bacillus thuringiensis* subsp. *kurstaki* Berliner; commonly abbreviated as *Bt*) are currently the insecticides of choice for most forest pest control operations in Canada (van Frankenhuyzen 1993). *Bt* is generally considered to be an environmentally safe alternative to chemical insecticides because: it has an extremely low toxicity to vertebrate animals, including birds, in comparison to traditional chemical insecticides (Burgess *et al.* 1995); and the protein crystal toxin of *B.t.k.* is highly specific to larval Lepidoptera so that natural enemies of forest pests and other non-lepidopteran beneficial insects are unaffected by forest spraying (Barber *et al.* 1995; van Frankenhuyzen 1995).

Tebufenozide is an example of a new class of insecticides, the dibenzoyl hydrazines, that show promise as forest pest control agents. Tebufenozide has a novel mode of action. When ingested by lepidopteran larvae, tebufenozide behaves similarly to the natural ecdysteroid 20-hydroxyecdysone, binding to the ecdysone receptor and inducing a precocious moult (Retnakaran *et al.* 1995). Within a few hours of treatment, the affected larvae stop feeding, remain moribund for several days and eventually die. Tebufenozide appears to be specific to Lepidoptera, even though ecdysteroids and their receptors are present in all orders of insects (Sohi *et al.* 1995).

Concerns have been raised about the possible indirect effects of forestry and agricultural insecticides on birds and other wildlife (DeWeese *et al.* 1979; Powell 1984; Hunter & Witham 1985; Yahner, Quinn & Grimm 1985; Spray, Crick & Hart 1987; Cooper *et al.*

1990; Millikin & Smith 1990; George, McEwen & Fowler 1992; Sample, Cooper & Whitmore 1993; Whitmore, Cooper & Sample 1993; Anonymous 1994; Burgess *et al.* 1995; Fair, Kennedy & McEwen 1995). Insectivorous forest songbirds may be particularly vulnerable to such effects, because forest spraying usually takes place in mid-spring when insect populations are high and the energy requirements of breeding songbirds are greatest (Cooper *et al.* 1990; Sample, Cooper & Whitmore 1993). During the breeding season, caterpillars are a particularly important component of the diet of many forest passerines (Mitchell 1952; Robinson & Holmes 1982; Holmes & Schultz 1988). Depressing caterpillar abundance could indirectly affect reproduction by preventing adults from obtaining enough food for their young or by altering parental behaviour (Powell 1984).

This study was designed to test the hypothesis that insecticide-induced reductions of Lepidoptera prey would affect Tennessee warbler *Vermivora peregrina* Wilson nest behaviour and reproduction. The study was part of a larger project undertaken co-operatively by the Canadian Forest Service, the Canadian Wildlife Service and the University of Toronto to investigate the indirect effects of forest spraying with *Bt* and tebufenozide (MIMIC) on songbird behaviour, diet, energetics and reproduction.

Materials and methods

The study was conducted about 15 km north-west of Longlac, Ontario, Canada (49°46′N, 86°33′W). Treatments were randomly assigned among blocks (each about 300 ha in size). One of the treated blocks was sprayed on the morning of 12 June 1994 with *Bt* (Foray 76B; Novo Nordisk Bioindustrials, Inc., Danbury, CT, USA) at a rate of 30 BIUs (Billion International Units) in a total volume of 1·5 L ha^{-1}. The other block was treated twice, on the morning of 14 June and again on the evening of 18 June 1994, with tebufenozide (MIMIC 240 LV; Rohm and Haas Canada Inc., West Hill, Ont., Canada) at a rate of 70 g of active ingredient in 2·0 L ha^{-1}. The applications were made using a team of two Cessna 188 aircraft, each of which was equipped with four Micronair® AU 4000 spray atomisers. The planes used AGNAV® GPS Guidance Systems (PICODAS Group Inc., Richmond Hill, Ont., Canada) to navigate the flight lines. The *Bt* formulation was applied 'neat' (i.e. no added diluent) and dyed with Erio Acid red dye (0·01% V/V) to facilitate deposit assessment. The MIMIC formulation was diluted with water and contained 0·01% V/V Rhodamine WT dye. The control block was not sprayed.

All three blocks were selected to be as similar as possible, but there was some variation. The MIMIC block was in an area of glaciolacustrine lake plain deposits, containing a high percentage of surface sand and silt (Gartner 1979). Soil drainage varied from good to poor, and the site was characterized by a mixture of small, boggy areas, interspersed with drier areas. The control and *Bt* blocks were on outwash plains (Gartner 1979), and were not as poorly drained. Soil textures here ranged from fine sand to coarse gravel. The local relief of all three sites was mainly low.

The study blocks were characterized, in varying degrees, by mixed stands of trembling aspen *Populus tremuloides* Michx., balsam fir *Abies balsamea* (L.) Mill., white spruce *Picea glauca* (Moench) Voss, black spruce *P. mariana* (Mill.) BSP. and white birch *Betula papyrifera* Marsh. Present to a lesser degree were jack pine *Pinus banksiana* Lamb., tamarack *Larix laricina* (DuRoi) K. Koch, eastern white cedar *Thuja occidentalis* L., alder *Alnus rugosa* (Du Roi) Spreng. and *A. crispa* (Ait.) Pursh, mountain maple *Acer spicatum* Lam., willow *Salix* sp. and mountain ash *Sorbus* sp. Common understory species included bunchberry *Cornus canadensis* L., grasses (Poaceae), large-leaved aster *Aster macrophyllus* L., sweet coltsfoot *Petasites palmatus* (Ait.) Gray., sarsaparilla *Aralia nudicauli*s L., wintergreen *Pyrola* spp., clubmosses *Lycopodium* spp., wild currant *Ribes* sp., bracken fern *Pteridium aquilinum* (L.) Kuhn and Labrador-tea *Ledum groenlandicum* Oeder. All three blocks were underlain with a thick carpet of mosses and lichens, including *Pleurozium schreberi* (Brid.) Mitt., *Hylocomium splendens* (Hedw.) BSG., *Brachythecium salebrosum* (Web. & Mohr) BSG., *Ptilium crista-castrensis* (Hedw.) De Not., *Sphagnum* spp., *Cladina rangiferina* (L.) Harm. and *Cladonia* spp.

Tennessee warblers were studied for three reasons: they were abundant in the study area; they are important predators of spruce budworm (Kendeigh 1947; Mitchell 1952; Morris *et al.* 1958); and, being ground-nesters, their nests were relatively easy to find and monitor. Nests were located by following females, observing their behaviour and listening for vocal cues. A total of six Tennessee warbler nests containing either eggs or nestlings were located in the MIMIC block, six in the control block and two in the *Bt* block. One of the nests in the MIMIC block was almost immediately abandoned and was not included in the data analysis.

During the egg laying and incubation stages, Tennessee warbler nests were visited daily and eggs were counted. On the day it hatched, each nestling was weighed on a Pesola® balance, its left tarsus was measured using a pair of dividers and a ruler, and it was given a unique combination of marks on the wings and/or legs, using a Sanford® non-toxic, permanent marker. These marks were replaced by coloured leg bands when the nestlings were 3–5 days old. During the nestling stage, nest visits and nestling measurements were made less frequently, usually every second day, to reduce the amount of disturbance.

On the days that nestlings were not measured, those nests were videotaped using a Sony® CCD-TR30

Video 8 Handycam®. The camera was mounted on a tripod about 1–2 m from the nest, and the camera and tripod were concealed using camouflage cloth to make the apparatus less obvious to parents and potential predators. Videotaping was done daily, usually between 8·00 and 11·00 h (early sessions) and between 11·00 and 14·00 h (late sessions), beginning on 29 June and ending on 6 July. With three cameras, it was possible to videotape a maximum of six nests each day. Videotaping sessions for each nest were alternated between early and late time periods, with 2–4 days (median 2 days) between taping sessions for any particular nest. Sessions usually lasted about 2 h (maximum tape length), although some were shorter due to technical difficulties. Over the 8-day study period in the MIMIC and control blocks, 36 sessions were taped for a total of 69·7 h. Too few tapes were made in the *Bt* block to warrant analysis.

Videotapes were played back through the camera on a Panasonic® Colour Video Monitor, Model No. CT-1930VC. Behaviour patterns of parents were classified (e. g. brooding, perching, nest cleaning, feeding nestlings, etc.), timed and counted. Prey items brought back to nestlings were identified, counted and their size was estimated relative to the adult beak length (10·5 mm; Godfrey 1966).

Data were analysed using BMDP/Dynamic Release 7·0 for the PC (BMDP Statistical Software, Inc., Los Angeles, CA). Separate variance (Welch) *t*-tests were used to identify differences between two groups. Differences among more than two groups, and interactions between grouping factors, were assessed by analysis of variance (ANOVA), followed by planned, multiple comparisons. For ANOVA, data that failed Levene's test (Brown & Forsythe 1974a) were transformed to satisfy the assumption of equality of group variances. If a suitable transformation could not be found, a robust test was used (Brown & Forsythe 1974b) rather than the standard ANOVA. Data that were not normally distributed were analysed by ANOVA on ranks. In follow-up multiple comparison tests, the experiment-wise error rate was controlled by adjusting the significance level of the Welch *t*-tests for the number of comparisons in each analysis (Bonferroni adjustment; Day & Quinn 1989).

Results

The first partially constructed nest was found on 8 June. Of the 13 nests monitored in this study, two were located while they were being built, two were at the egg-laying stage, seven were at the incubation stage and two contained nestlings. From nest observations, it was possible to construct an approximate nesting chronology for Tennessee warblers in this study. Laying occurred from 17 to 22 June, incubation from 20 to 30 June and hatching from 27 June to 1 July. The median hatch date was 29 June.

Nests in the treated blocks (MIMIC and *Bt*) contained fewer eggs and nestlings than those in the control block (Table 1), but these differences were not significant (Welch *t*-tests: MIMIC vs. control eggs, $t = 1{\cdot}37$, $P = 0{\cdot}2075$; MIMIC vs. control nestlings, $t = 1{\cdot}59$, $P = 0{\cdot}1460$; *Bt* vs. control eggs, $t = 1{\cdot}00$, $P = 0{\cdot}3632$; *Bt* vs. control nestlings, $t = 0{\cdot}96$, $P = 0{\cdot}4032$). In addition, hatch rates in the treated blocks were lower than in the control block (Table 1). Again, the differences were not significant (likelihood ratio chi-square: MIMIC vs. control, $\chi^2 = 0{\cdot}054$, $P = 0{\cdot}8136$; *Bt* vs. control, $\chi^2 = 0{\cdot}016$, $P = 0{\cdot}8993$).

Average nestling weights were generally lower in the *Bt* block than in the control block (Fig. 1), but this difference was significant only at age 3 days (Welch *t*-test: $t = 3{\cdot}35$, $P < 0{\cdot}05$). The only other significant differences observed were the weight of MIMIC vs. control nestlings at age 4 days (Welch *t*-test: $t = -3{\cdot}56$, $P < 0{\cdot}05$) and the tarsus length of *Bt* vs. control nestlings at age 5 days (Welch *t*-test: $t = -3{\cdot}97$, $P < 0{\cdot}01$). In both of these cases, nestling measurements were greater in the treated blocks than in the control block (Fig. 1).

Growth rates of MIMIC, *Bt* and control nestlings were calculated by fitting the age-weight data illustrated in Fig. 1 to a series of logistic growth curves (Ricklefs 1967). MIMIC nestlings had the fastest growth rate [linear regression: $r^2 = 0{\cdot}9867$, $P < 0{\cdot}0001$, K (rate constant of the growth equation $= 0{\cdot}6776$)]; control nestlings had the slowest growth rate ($r^2 = 0{\cdot}9682$, $P = 0{\cdot}0001$, $K = 0{\cdot}5580$); and *Bt* nestlings had an intermediate growth rate ($r^2 = 1{\cdot}0000$, $P < 0{\cdot}0001$, $K = 0{\cdot}5900$). However, these differences were not significant (analysis of variance of regression coefficients over groups: d.f. = 4,12, $F = 2{\cdot}72$, $P = 0{\cdot}0802$).

Rodenhouse & Holmes (1992) suggested that, in cases where food limits growth, developmental effects might be manifested in only one or two nestlings in a brood. If this is the case, then using means to calculate growth rates, as was done above, could mask within-brood differences in nestling growth attributable to treatment. To compensate, individual growth rates were calculated for nestlings whose weights were measured on at least 3 days, and these individual growth rates were then used to calculate an average growth rate for the nestlings on each block [$1{\cdot}31 \pm 0{\cdot}13$ g day^{-1} (mean ± standard deviation, $n = 26$) for MIMIC nestlings; $1{\cdot}18 \pm 0{\cdot}09$ g day^{-1} ($n = 30$) for control nestlings; $1{\cdot}21 \pm 0{\cdot}08$ g day^{-1} ($n = 5$) for *Bt* nestlings]. As in the previous analysis, MIMIC nestlings grew fastest and control nestlings slowest, but in this case the difference was significant (Welch *t*-test: $t = -4{\cdot}40$, $P < 0{\cdot}0005$). There was no significant difference between *Bt* and control nestlings, however (Welch *t*-test: $t = -0{\cdot}72$, $P = 0{\cdot}4977$). The same results were obtained when the analysis was restricted to the two slowest growing nestlings in each brood; MIMIC nestlings grew significantly faster than control nestlings ($1{\cdot}24 \pm 0{\cdot}04$ g day^{-1} vs. $1{\cdot}10 \pm 0{\cdot}06$ g

Table 1. Mean (standard deviation; total) numbers of Tennessee warbler eggs and nestlings in nests in the two treated blocks (*Bt* and MIMIC) and in the untreated control block

	Nests	Eggs	Nestlings	% Hatch*
Control	6	6·3 (0·8; 38)	6·2 (1·2; 37)	97·4
Bt	2	6·0 (0·0; 12)	5·5 (0·7; 11)	91·7
MIMIC	5	5·8 (0·4; 29)	5·2 (0·8; 26)	89·7
Treated	7	5·9 (0·4; 41)	5·3 (0·8; 37)	90·2

* % Hatch = (total no. of nestlings/total no. of eggs) × 100.

day^{-1}, Welch *t*-test: $t = -6{\cdot}14$, $P < 0{\cdot}0001$), but *Bt* nestlings did not ($1{\cdot}13 \pm 0{\cdot}04$ g day^{-1}, Welch *t*-test: $t = -0{\cdot}77$, $P = 0{\cdot}5124$).

The only nestling mortality in the study occurred as a result of predation. One nest in the control block was depredated when the nestlings were 4 or 5 days old; and one nest in the *Bt* block was depredated when the nestlings were < 1 day old.

Parental behaviour patterns at the nest were not analysed for *Bt*. Only two sessions totalling 3·5 h were taped at the two nests on this block.

There was no significant difference between blocks (MIMIC and control) in the frequency of visits to the nest by Tennessee warbler parents (two-way ANOVA: block, d.f. = 1,67, $F = 0{\cdot}06$, $P = 0{\cdot}8001$). On both MIMIC and control blocks, females made more frequent visits to the nest than did males, but the difference was not significant (two-way ANOVA: sex, d.f. = 1,67, $F = 2{\cdot}11$, $P = 0{\cdot}1513$). Females made on average $5{\cdot}3 \pm 0{\cdot}6$ (mean ± standard error) visits hour^{-1} to the nest in the MIMIC block vs. $5{\cdot}1 \pm 0{\cdot}7$ visits hour^{-1} in the control block; males made $4{\cdot}5 \pm 0{\cdot}4$ visits hour^{-1} in the MIMIC block vs. $4{\cdot}3 \pm 0{\cdot}5$ visits hour^{-1} in the control block. The frequency of parental nest visits was unaffected by nestling age (one-way ANOVA: d.f. = 6,64, $F = 1{\cdot}44$, $P = 0{\cdot}2142$).

Intervals between visits to the nest by female parents were generally shorter in the control than in the MIMIC block and increased with increasing nestling age (Fig. 2; two-way ANOVA on ranks: block, d.f. = 1,299, $F = 4{\cdot}86$, $P = 0{\cdot}0283$; age, d.f. = 5,299, $F = 11{\cdot}51$, $P < 0{\cdot}0001$). However, for any particular age, the difference between blocks was not significant (Welch *t*-tests: $P > 0{\cdot}05$). The same pattern was not

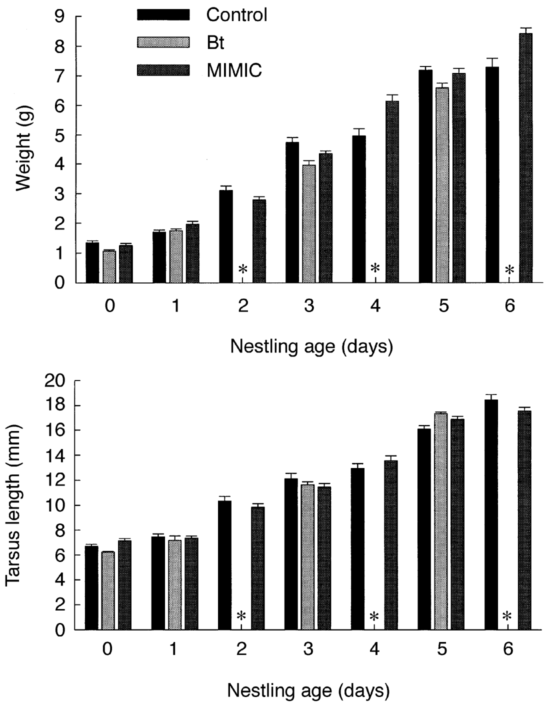

Fig. 1. Average weights and tarsus lengths of Tennessee warbler nestlings from day of hatch to age 6 days. *No data; error bars denote standard error.

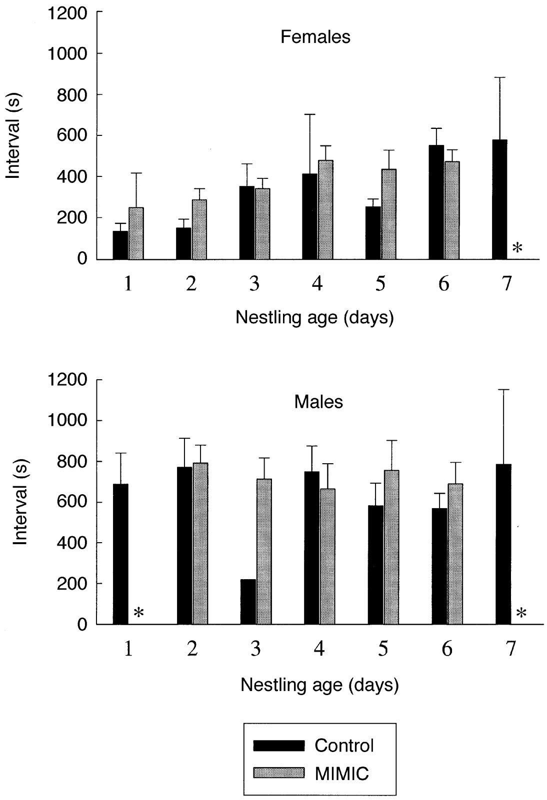

Fig. 2. Average interval in seconds between visits to the nest by adult female and male Tennessee warblers during the early nestling period (from age 1–7 days). *No data; error bars denote standard error.

seen among male parents (Fig. 2; two-way ANOVA on ranks: block, d.f. = 1,218, $F = 2·90$, $P = 0·0903$; age, d.f. = 4,218, $F = 1·42$, $P = 0·2273$).

When intervals between nest visits were grouped according to duration, with short intervals being <2 min and long intervals >2 min, there was a significant difference between blocks in the proportion of short to long intervals. For both male and female parents, the proportion of short intervals was greater in the control than in the MIMIC block (Table 2; likelihood ratio chi-square: females, $\chi^2 = 18·717$, $P < 0·0001$; males, $\chi^2 = 16·277$, $P = 0·0001$). The proportion of short intervals was also greater for females than for males (Table 2; likelihood ratio chi-square: control, $\chi^2 = 28·722$, $P < 0·0001$; MIMIC, $\chi^2 = 23·042$, $P < 0·0001$).

The proportion of time spent at the nest by female parents did not differ significantly between the MIMIC and control blocks (Fig. 3; two-way ANOVA: block, d.f. = 1,22, $F = 3·88$, $P = 0·0615$). It should be noted, however, that female parents did spend significantly less time at the nest with increasing nestling age (Fig. 3; two-way ANOVA: age, d.f. = 5,22, $F = 10·11$, $P < 0·0001$). There were no significant differences between blocks or among nestling ages in the proportion of time spent at the nest by male parents (two-way ANOVA, log transformed data: block, d.f. = 1,20, $F = 3·95$, $P = 0·0608$; age, d.f. = 4,20, $F = 1·31$, $P = 0·2989$).

Table 2. Proportion of long (>2 min) vs. short (<2 min) intervals between nest visits by adult Tennessee warblers during the early nestling period (from age 1–7 days)

	Proportion of intervals (%)	Short	Long
Female	Control	41·5	58·5
	MIMIC	19·4	80·6
Male	Control	15·2	84·8
	MIMIC	1·8	98·2

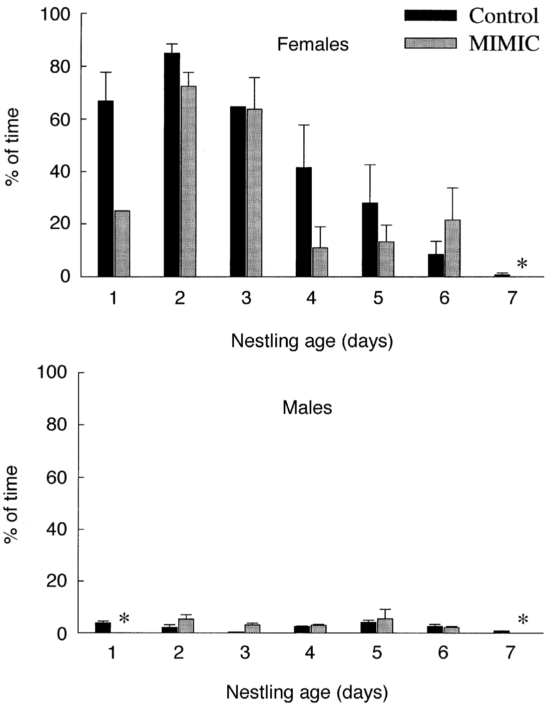

Fig. 3. Proportion (%) of time spent at the nest by adult female and male Tennessee warblers during the early nestling period (from age 1–7 days). *No data; error bars denote standard error.

Females spent significantly less time brooding young in the MIMIC block than they did in the control block (Fig. 4; two-way ANOVA: block, d.f. = 1,22, $F = 4·77$, $P = 0·04$). In the MIMIC block, brooding occupied 82·8% of the females' time at the nest, compared to 89·8% in the control. Feeding nestlings took an additional 7·9% of the females' time in the MIMIC block and 3·8% in the control. There was no difference between the MIMIC and control blocks in the amount of time that parents spent feeding nestlings (two-way ANOVA on ranks: block, d.f. = 1,22, $F = 0·39$, $P = 0·5405$ for females; block, d.f. = 1,20, $F = 3·44$, $P = 0·0786$ for males). Other behaviour patterns observed at the nest included nest maintenance (e. g. nest repair, cleaning, faecal sac removal) and perching (loafing).

The number of insects brought back to the nest by adult Tennessee warblers did not differ significantly between the MIMIC and control blocks, but did increase with increasing nestling age (Fig. 5; two-way ANOVA: block, d.f. = 1,46, $F = 0·52$, $P = 0·4739$; age, d.f. = 4,46, $F = 4·56$, $P = 0·0035$).

There were no significant differences between blocks (MIMIC and control) or among nestling ages in the size of insects brought back to the nest (two-way ANOVA: block, d.f. = 1,658, $F = 0·18$, $P = 0·6708$; age, d.f. = 4,658, $F = 1·53$, $P = 0·1908$). The average prey size (length) was $14·5 \pm 3·9$ mm (mean ± standard deviation). Caterpillars were by far the predominant food of nestlings in both blocks,

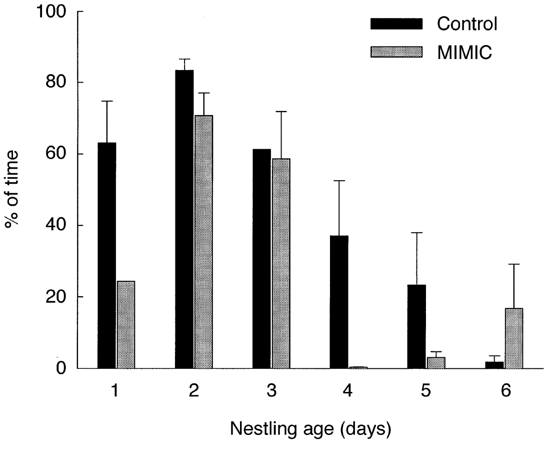

Fig. 4. Proportion (%) of time spent brooding by adult female Tennessee warblers during the early nestling period (from age 1–6 days). Error bars denote standard error.

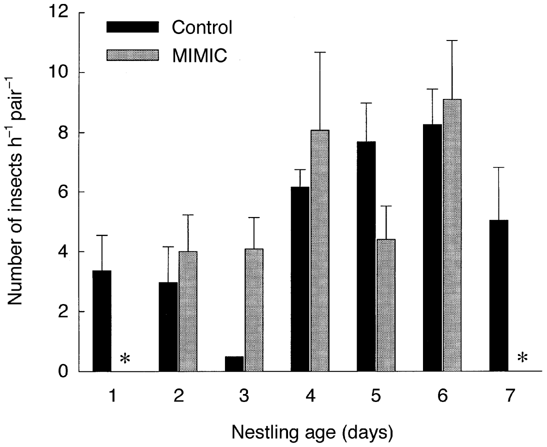

Fig. 5. Average number of insects brought back to the nest by adult Tennessee warblers during the early nestling period (from age 1–7 days). *No data; error bars denote standard error.

making up 91·1% of food items in the control block and 93·2% in the MIMIC block. This proportion did not differ significantly between blocks (likelihood ratio chi-square: $\chi^2 = 1{\cdot}108$, $P = 0{\cdot}2925$).

Discussion

Because *Bt* and MIMIC insecticides are not directly toxic to vertebrates (Burgess *et al.* 1995; Rohm and Haas Company, unpublished data), any effects observed in the present study could only have resulted from reductions in the birds' invertebrate food supply. Changes in Lepidoptera populations in the MIMIC, *Bt* and control blocks were measured in a separate study. In the MIMIC block, lepidopterans on spruce (including spruce budworm) and aspen were reduced in numbers relative to the control by more than 90% and about 77%, respectively (Holmes *et al.* 1997). In contrast, lepidopterans on spruce in the *Bt* block were not reduced in numbers and lepidopterans on aspen were reduced by only about 15% (Holmes *et al.* 1997). From these results, it appears that the MIMIC spray was very effective, but the *Bt* spray was not. Considering its poor efficacy (lack of direct effects), it is unlikely that the *Bt* spray could have caused any indirect effects on forest songbirds.

The reasons for the poor efficacy of the *Bt* spray are unclear. Spray deposit assessment on Kromekote® cards placed on the ground in the *Bt* block revealed a deposit of 7·12 drops cm^{-2}, which is considered good for this type of application (L. Cadogan, Canadian Forest Service, personal communication). However, the *Bt* application was followed by two days of rain, which may have washed much of the insecticide from the foliage. The MIMIC applications were unaffected by rain.

On 3 July, when nestlings were 3–6 days old (median 3 days), lepidopteran densities on spruce were considerably lower in the MIMIC block (0·54 Lepidoptera/45-cm branch) than in the control block (5·14 Lepidoptera/branch, Holmes *et al.* 1997). On aspen, lepidopteran numbers were only slightly lower in MIMIC (1·06 Lepidoptera/500 leaf bundles) than in control blocks (1·58 Lepidoptera/500 leaf bundles; Holmes *et al.* 1997). Despite the lower amount of food available in the MIMIC block, these nestlings had the fastest growth rate (about 11–21% faster than control nestlings and 8–15% faster than *Bt* nestlings, depending on how growth rate was calculated). This apparent discrepancy can be explained by differences in brood size. Tennessee warblers in the control block had the largest broods (average 6·2 nestlings), while average brood sizes in the MIMIC and *Bt* blocks were 19% and 13% smaller (5·2 and 5·5 nestlings), respectively. Since there were no differences between blocks (MIMIC and control) in the number or size of prey items brought back to the nests by parents, it follows that nestlings in the MIMIC block would have received more food than nestlings in the control block, resulting in more rapid growth. Feeding rates of nestlings were not measured in the *Bt* block.

The reasons for the observed differences between blocks in clutch size, brood size and hatching success are unclear. Although the trends suggest a treatment effect, the differences between blocks were small and not statistically significant. Similarly, Spray, Crick & Hart (1987) found no significant differences in the clutch size or brood size of coal tits *Parus ater* L. in forests treated with fenitrothion insecticide vs. unsprayed forests.

Tennessee warblers, like bay-breasted warblers *Dendroica castanea* Wilson and cape may warblers *Dendroica tigrina* Gmelin, are thought to increase their clutch size in response to outbreaks of spruce budworm (Morse 1989). For example, MacArthur (1958) demonstrated that bay-breasted warbler clutches in north-eastern New Brunswick were larger during years of budworm outbreaks than during non-budworm years. If the factors controlling clutch size in Tennessee warblers operate at a local level and not just at the landscape level described by MacArthur (1958), then one might have expected larger clutches in the MIMIC block due to its high prespray budworm population (46·0 budworm/45-cm spruce branch vs. 18·5 budworm/branch in the control block and only 6·1 budworm/branch in the *Bt* block; Holmes *et al.* 1997). In fact, the MIMIC block had the smallest average clutch size, and the control block the largest. This suggests that some other factor (s) was responsible for the observed differences in clutch size between blocks. Klomp (1970) lists the proximate factors involved in determining clutch size. In addition to food, these include age of female, weather, habitat and population density, any or all of which might have been operating in the study area.

There were no significant differences in hatching success between the control and treated blocks, although there was a trend suggesting lower hatch rates in the MIMIC and *Bt* blocks. A number of other field studies have failed to demonstrate any relationship between insecticide treatment and hatchability of eggs (Powell 1984; Spray, Crick & Hart 1987; Robinson *et al.* 1988; Busby, White & Pearce 1990; Pascual & Peris 1992; Pascual 1994).

In considering between-block differences in clutch size, brood size and hatching success, it is important to keep in mind the small number of nests monitored in this study and the resulting low power of the statistical tests comparing blocks. Due to small sample sizes, real differences between blocks, as opposed to statistical differences, may not have been detected. With this in mind, it is suggestive, although not necessarily compelling, that reproductive parameters were consistently lower in the treated blocks than in the control block.

In two respects, the behaviour of female Tennessee warblers in the MIMIC block differed significantly from that in the control block. The MIMIC females spent less time brooding than control females and their foraging trips away from the nest were longer in duration. These two behaviours would appear to be linked in the following way: if locating and capturing prey were more difficult in the MIMIC block after spraying due to reduced prey populations, then one would expect foraging times to increase; with more time spent foraging, less time would be available for other activities, including brooding.

The nest behaviour of male Tennessee warblers was unaffected by the MIMIC treatment. This may be related to the fact that males spend much less time caring for the young than do females (ratio of about 13:1 for females vs. males). Male Tennessee warblers may have compensated for increased time spent foraging by reducing other activities (e. g. territorial defence) that were not measured in this study.

There were no differences in nestling diets between the MIMIC and control blocks, despite the fact that Lepidoptera on spruce were almost 10 times scarcer in the MIMIC block than in the control block by the end of the study. Tennessee warblers are budworm specialists, and although it was not always possible to discriminate between budworm and other lepidopterans when viewing the videotapes, it did appear that birds in the MIMIC block continued to collect budworm larvae and pupae after spraying. It is possible that birds from the MIMIC block may have foraged outside the sprayed area, but this is unlikely, given the large size of the block (about 300 ha). A more reasonable explanation might be that birds were finding budworm in locations or 'refugia' that did not receive spray deposit. For example, E. Ketella (Canadian Forest Service, personal communication) suggests that, due to the fact that insecticide is deposited mostly in the upper canopy, budworm may persist in lower strata, either in the lower parts of the canopy or in smaller trees, at relatively high numbers after forest spraying. Pole-pruning, the method used to collect lepidopterans in this study, did not sample these lower strata. Refugia may also be created if there are gaps in the spray coverage. An examination of the output from the aircrafts' guidance systems did reveal a few gaps, but they were small and dispersed across the block. Overall, spray coverage of the MIMIC block exceeded 90% for both applications (A. Robinson, Canadian Forest Service, personal communication).

In the only other published study directly comparable to this one, Rodenhouse & Holmes (1992) examined the effects of *Bt*-induced reductions in food abundance on the reproductive ecology of black-throated blue warblers *Dendroica caerulescens* Gmelin. They found that *Bt* treatment did not affect clutch size, hatching success, fledging success or annual breeding productivity, but that second broods were less common as a result of reduced caterpillar abundance. No attempt was made in the present study to determine the frequency of second broods by Tennessee warblers. The timing of Tennessee warbler nesting appears to be highly synchronized with budworm development and is thus relatively late in the season compared to black-throated blue warblers [e. g. first egg date of 17 June for Tennessee warblers in this study vs. mean first egg date of 2 June for black-throated blue warblers in Rodenhouse & Holmes' (1992) study]. This late timing enables Tennessee warbler parents to take advantage of a super-abundant food source (i.e. large budworm larvae and pupae) for rearing their young. It probably also limits them to producing single broods.

Rodenhouse & Holmes (1992) also reported that nestling diets contained significantly fewer caterpillars when caterpillar abundance was reduced by *Bt* spraying. This result is contrary to that observed for Tennessee warblers, where nestling diets were unaffected by MIMIC treatment. This difference between studies does not appear to be related to differences in efficacy. Rodenhouse & Holmes (1992) reported reductions in biomass of clinging arthropods ranging from about 60 to 80%. In the present study, numbers of caterpillars and pupae were reduced by more than 96%. While these data are not strictly comparable, they do suggest that the MIMIC spray was as effective, or possibly even more so, than Rodenhouse & Holmes' (1992) *Bt* spray.

The difference in nestling diets between this and the above-mentioned study is probably due to differences in the foraging habits of black-throated blue and Tennessee warblers. Rodenhouse & Holmes' (1992) study was conducted in a second-growth northern hardwood forest, where black-throated blue warblers typically forage by hover-gleaning prey from leaves in the deciduous understory (Holmes, Bonney & Pacala 1979; Robinson & Holmes 1982). In contrast, Tennessee warblers inhabit boreal and mixed-boreal forests, and during periods of outbreak are extreme budworm specialists (Mitchell 1952). If alternative sources of prey (non-Lepidoptera) are readily available in the foraging niche of black-throated blue warblers, then one might expect them to respond to reduced caterpillar abundance by diversifying their diets. On the other hand, Tennessee warblers may be less flexible in their foraging habits and continue to seek budworm prey even when populations are severely reduced.

In a comparable study, Pascual & Peris (1992) investigated the indirect effects of forest spraying with the synthetic pyrethroid insecticide cypermethrin on the breeding success of blue tits *Parus caeruleus* L. Although synthetic pyrethroids are broad spectrum insecticides, they are not very toxic to vertebrates (Smith & Stratton 1986). Thus, any effects on songbirds should be indirect. In Pascual & Peris' (1992) experiment, two treatment blocks were sprayed with cypermethrin at different application rates. In the block that received the lower rate, they found that caterpillars were reduced by about 90% (a level comparable to the present study), and that nestlings in late nests suffered higher rates of mortality than control nestlings. Other breeding parameters (nest abandonment, nest success, hatching success, nestling weight) were unaffected, however. In the higher rate block, which suffered almost complete mortality of lepidopteran larvae, nestling survival, nest success and nestling weight were all significantly reduced.

The effects documented by Pascual & Peris (1992), even at the lower application rate, were more severe than those observed in the present study. The reasons for this difference are unclear, but they may be related to differences in prey densities or songbird foraging patterns. As noted previously, Tennessee warblers are considered to be extreme budworm specialists in terms of their dietary habits (Mitchell 1952). It could be that Tennessee warblers are so efficient in searching for their preferred prey that they are able to exploit very low prey (budworm) populations, something a generalist predator would not be able to do.

There have been few other studies of the potential indirect effects of insecticides on birds where direct toxicity was not a complicating factor. Most of these have involved the insecticide Dimilin®. For example, spraying forests with Dimilin® to control gypsy moth has been shown to affect songbird diets (Cooper *et al.* 1990; Sample, Cooper & Whitmore 1993). Since Dimilin® has an extremely low vertebrate toxicity (Maas *et al.* 1981), these changes were attributed to a decrease in insect prey abundance on the treated plots. Dimilin® spraying has also been shown to affect foraging behaviour (Cooper *et al.* 1990) and fat levels (Whitmore, Cooper & Sample 1993) in adult birds, but not breeding success or nestling growth (de Reede 1982). In studies of insecticides that are directly toxic to birds (e. g. Powell 1984; Busby, White & Pearce 1990; Millikin & Smith 1990), it is more difficult to differentiate between direct and indirect effects.

Conclusions

In drawing conclusions based on the results of this study, it is important to keep in mind the following limitations. First, and most importantly, there was no replication of experimental treatments. As a consequence, the differences observed between blocks in the post-spray period cannot be unequivocally ascribed to insecticide effects. As stated previously, an attempt was made to select blocks that were as similar as possible and to assign treatments to the blocks randomly. However, prior to spraying, no information was available on the Lepidoptera populations. Subsequent analyses revealed large pre-spray differences between blocks (i.e. 61·1 Lepidoptera/45-cm spruce branch on MIMIC, 10·5 Lepidoptera/branch on *Bt* and 24·2 Lepidoptera/branch on control; Holmes *et al.* 1997), differences that would no doubt have influenced predator behaviour. This problem might have been avoided by assigning several blocks to each treatment, either randomly or stratified by pest population, so that pretreatment block differences were eliminated or at least minimized. Unfortunately, due to resource constraints, this was not a viable option.

Additionally, the small sample sizes in this study mean that the power of statistical tests was low. Finding an adequate sample of natural nests to conduct nesting studies can be difficult. Others have solved this problem by studying species that construct conspicuous nests in relatively high densities (see Powell 1984) or by using artificial nest boxes (see Spray, Crick & Hart 1987). Neither of these two options was possible in the present study.

Bearing in mind the foregoing, the following observations can be made. Results from this study are consistent with the hypothesis that forest spraying with Lepidoptera-specific insecticides can indirectly affect forest songbirds. Specifically, female Tennessee warblers altered their foraging behaviour to compensate for reduced lepidopteran populations in the MIMIC-treated block, which in turn led to reduced nest attentiveness (brooding). However, this reduction in parental care was not sufficient to affect nest success or nestling growth.

Considering these results together with those from other studies, it can be concluded that pest control programmes using Lepidoptera-specific insecticides, such as *Bt* and MIMIC, pose relatively little threat to songbirds. The indirect effects observed following applications of these materials are small compared to the combination of direct and indirect effects resulting from exposure to traditional chemical insecticides. The choice of Lepidoptera-specific insecticides for use in pest control programmes, as opposed to insect-specific (e. g. Dimilin) or non-specific (e. g. organophosphate and carbamate) insecticides, represents a significant step forward in environmental protection.

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