Several bird species, including cavity-nesters such as European starlings *Sturnus vulgaris*, add to their nests green sprigs of plants such as yarrow *Achillea millefolium* that are rich in volatile compounds. In this field study on another cavity-nester, tree swallows *Tachycineta bicolor*, we tested whether yarrow reduced ectoparasite loads (the nest protection hypothesis), stimulated nestling immune systems (the drug hypothesis), or had other consequences for nestling growth or parental reproductive success (predicted by both preceding hypotheses). Tree swallows do not naturally add greenery to their nests, and thus offer several advantages in testing for effects of greenery independent of other potentially confounding explanations for the behaviour.

We placed fresh yarrow in 23 swallow nests on the day the first egg was laid, replenishing every two days until clutch completion (=three times), and at 44 control nests, nesting material was simply touched. At 12 days of age, we measured nestling body size and mass, and took blood smears to do differential white blood cell counts. We subsequently determined the number and proportion of young fledging from nests and the number of fleas remaining after fledging. Higher humidity was associated with higher flea numbers whereas number of feathers in the nest was not. Our most significant finding was that an average of 773 fleas *Ceratophyllus idius* was found in control nests, versus 419 in yarrow nests. Possibly, parents compensate for blood that nestlings lose to ectoparasites by increasing food delivery, because we detected no differences between treatments in nestling mass, nestling leukocyte profiles, or proportion of young fledging, or relative to flea numbers. Our results provide no support for the drug hypothesis and strong support for the nest protection hypothesis.

Several bird species add sprigs of green vegetation (hereafter, greenery) to their nests (Wimberger 1984, Clark 1990, 1991). Among six explanations for this behaviour offered by Clark (1991), the one he favoured was that birds preferentially select plant species containing volatile chemicals that have insecticidal effects on nest ectoparasites (nest protection hypothesis). For example, greenery is used more often by species that reuse their nests; reused nests may be more susceptible than newly-built nests to the accumulation of ectoparasites (Wimberger 1984, Clark and Mason 1985). Similarly, Clark (1991) showed that mite populations were significantly higher in nests of European starlings *Sturnus vulgaris* (hereafter starlings) if greenery was experimentally removed. In this paper, our principal objective was to test whether one of the plants that starlings incorporate in their nests, yarrow *Achillea millefolium*, reduces ectoparasite populations.

Gwinner et al. (2000) found no difference in ectoparasite populations between artificial control (grass) and experimental greenery (herb) starling nests. Because some of their results were not consistent with the nest protection hypothesis, they proposed instead that greenery enhanced nestling immunity to cope with ectoparasites (drug hypothesis). Accordingly, they found higher haematocrits, proportions of basophils, and growth rates in experimental nests, although they found no influence on immunocompetence. With the exception of their results for ectoparasite loads, Gwinner et al. (2000) noted that their other results were consistent with the nest protection hypothesis. For instance, even if ectoparasite numbers are
not affected, volatile compounds could affect ectoparasite feeding (Clark and Mason 1988), leading to nestlings with different leukocyte profiles, higher haematocrits, and higher growth rates. Regardless, both the nest protection and drug hypotheses assume that greenery enhances parental fitness, perhaps by reducing allocation of incubating parents and nestlings to immune function so that nestlings have more allo- cate to growth and survival. We do not consider here other hypotheses listed by Clark (1991) because they have limited support.

Because even experimental removal of greenery from starling nests necessarily follows an interval during which ectoparasites are exposed to volatile chemicals, we chose to test the nest protection and drug hypotheses using a species that does not normally incorporate greenery in its nests, the tree swallow Tachycineta bicolor. Moreover, because a mate choice function for greenery has been suggested for starlings (Fauth et al. 1991), tree swallows offer a significant advantage over starlings in the execution of control treatments because our tests may be freed of this and other competing explanations for this behaviour.

Tree swallows use artificial cavities for breeding, and are extremely tolerant to a variety of experimental manipulations (Robertson et al. 1992). We added greenery to tree swallow nests, and compared reproductive success, haematological and morphological parameters of nestlings, and ectoparasite populations in nests. Two other variables hypothesized to reduce fleas loads are higher numbers of feathers lining nests (Winkler 1993), and lower humidity (Heeb et al. 1998). Accordingly, we tested for associations between these covariates and flea loads.

**Methods**

All methods were approved by the Acadia University Animal Care Committee. We studied tree swallows at four freshwater impoundments in Nova Scotia and New Brunswick, Canada (between 45°40’N and 46°0’N, and 64°10’E and 64°30’E) from 22 May to 2 August 2000. At these impoundments, 141 nestboxes (in Nova Scotia, 43 at River Hebert, 31 at McIver Marsh, 31 at Eddy Road Marsh; in New Brunswick, 36 at Jolicure) had been in operation at least two years prior to the 2000 field season. Boxes were erected on poles 1.5 m above ground, spaced 30 m apart, and faced the impoundments. Old nesting material was removed from boxes in early April 2000.

Nest boxes were visited and checked every two days until fledging. We recorded clutch initiation date (assuming one egg was laid per day), clutch size (number of eggs), number of nestlings (at 16 days of age), and number fledging (number of nestlings less any dead found in the box after fledging).

Because it was locally abundant, we chose yarrow as the greenery to add to nests; it was collected from an undisturbed field that was not treated with pesticides adjacent to the Sackville Waterfowl Park. Before swallows arrived, ten boxes were randomly assigned to the yarrow treatment at each impoundment. If egg-laying began in these boxes, 5 g of yarrow were added to these nests three times over a six-day period until clutch completion or shortly thereafter; control nest boxes were opened and nestling material was touched.

When nestlings were 16 days old, we measured with callipers to the nearest 0.1 mm head length and tarsus bone (Dzubin and Cooch 1992). Unflattened wing length was measured with a wing ruler to the nearest 0.5 mm from the wing joint to the tip of the longest primary feather. Tail length was measured with a ruler to the nearest 0.5 mm from the middle of the tail to the end of the longest feather. Mass was measured to the nearest 0.5 g using a zeroed spring scale.

The brachial artery of the lightest and heaviest nestlings in each nest was punctured with a sterile lancet to make blood smears on microscope slides (Bennett 1970). Slides were stained using Hema 3 (Biochemical Sciences Inc.), and white blood cell counts were made using a microscope at 1000 power (Lucas and Jamroz 1961). For each smear, we counted 200 white blood cells (lymphocytes, monocytes, heterophils, eosinophils, basophils, and thrombocytes).

Nests and all detritus were removed from boxes and placed in separate bags within a week of fledging. The fleas Ceratophyllum idius that infest tree swallow nests overwinter as adults, but are killed if nests are frozen at −16°C for 2 weeks, removed for two days, and refrozen. Subsequently, nests were thawed, weighed, and dried at 150°C for 48 h, and reweighed to measure nest humidity (= (wet mass − dry mass)/dry mass). Nests were sifted to count feathers, fleas, and blowfly pupae. Because fleas were so numerous in nests, we divided the first 10 nests into halves, and counted each half separately. Flea estimates from separate halves of nests did not differ by more than 8%, so fleas were counted from only half of the material in the remaining 57 nests; blowfly pupae were still counted for the entire nest.

Statistical analysis was carried out using SAS (SAS Institute Inc. 1990), and means are reported ± SD. Growth (indexed by day-16 size and day-16 mass) and blood parameters were averaged for a brood so that nests were the unit of analysis, but because growth can be slower in larger broods (Shutler et al. 2006), we included brood size as a class random effect in mixed models comparing treatment and experimental nests. To reduce the number of tests and our experimentwise error rate, we used principal components (PC) analysis.
on the correlation matrix of head, tarsus, wing, and tail to create a single PC called SIZE (eigenvalue 2.5, 63% of variation explained, which is more than expected by chance using the broken stick criterion; Jackson 1993). Remaining PCs explained less variation than expected by chance and were not retained. Following Edwards et al. (2006), we used another PC analysis to create from the six blood cells enumerated a single blood profile parameter, LEUKOCYTES (eigenvalue 2.5, 42% of variation explained; remaining PCs were not significant and not retained).

### Results

#### General observations

We excluded from analyses nests (two at Jolicure, one at River Hebert, one at Eddy Road, and three at McIver) that did not produce any fledglings because ectoparasites would have had less time for population growth. Tree swallows produced fledglings in 22 of 31 nestboxes at Eddy Road, 20 of 36 boxes at Jolicure, 11 of 31 nests at McIver, and 14 of 44 nests at River Hebert. First-egg dates ranged from 24 May to 10 June, and there were 8, 16, 33, 8, and 2 clutches of 4, 5, 6, 7, and 8, respectively (mean = 5.7 ± 0.94). Nest initiation dates did not differ between control (30 May ± 5.3 days) and yarrow nests (31 May ± 4.3 days; F = 0.6, P = 0.44). Successful nests had 1 to 8 nestlings (mean = 5.2 ± 1.2), and produced between 1 and 8 fledglings (mean = 5.0 ± 1.4).

#### Experimental results

Yarrow addition did not affect measures of reproductive success (clutch size, number of nestlings, number fledging), mass, LEUKOCYTES, or number of blowfly pupae (Table 1). However, in mixed models with brood size as a class random effect, nestlings were larger in control than in yarrow nests (Table 1); this result remained significant if we controlled for nest initiation date. The strongest pattern to emerge was that flea populations in control nests were approximately double those in yarrow nests (Table 1, Fig. 1). In a mixed model with site as a random effect, flea numbers were positively associated with nest humidity (F1,60 = 5.0, P = 0.03), were not associated with feather number (F1,60 < 0.1, P = 0.86), and were much higher in control nests (F1,60 = 16.2, P < 0.001).

Despite potential blood loss to fleas, their numbers were not associated with variation in SIZE, mass, or LEUKOCYTES in either control or yarrow nests (Table 2). Within control nests, there was a positive association between flea numbers and number of nestlings (also see Shutler et al. 2004), but the association with number fledging was not statistically significant and there was no relationship with proportion fledging.

### Discussion

Yarrow dramatically reduced the number of fleas in nests, consistent with the nest protection hypothesis. This is surprising given that we used only three additions of yarrow per nest, and that we did it only after laying began, whereas starlings may add yarrow many more times, and up to two weeks before laying (Gwinner 1997, Brouwer and Komdeur 2004). This may illustrate the potency of yarrow as an insecticidal plant, although we also note that Ceratophyllus idius may not have evolved defences against greenery. We did not find that yarrow affected leukocyte profiles, contrary to the drug hypothesis. In addition, despite high flea populations in some nests, no negative impacts were detected on nestling tree swallows (also see Shutler et al. 2004). Some of our results are in contrast to three previous studies that failed to affect ectoparasite loads with manipulations of greenery. First,

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Mean ± SD</th>
<th>Control</th>
<th>Yarrow</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>5.5 ± 1.0</td>
<td>6.0 ± 0.9</td>
<td>3.7</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Nestlings</td>
<td>5.1 ± 1.1</td>
<td>5.3 ± 1.4</td>
<td>0.3</td>
<td>0.60</td>
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<tr>
<td>Fledged</td>
<td>5.1 ± 1.1</td>
<td>5.0 ± 1.8</td>
<td>0.1</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>SIZE</td>
<td>0.3 ± 1.6</td>
<td>-0.6 ± 1.5</td>
<td>5.5</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Mass (g)</td>
<td>23.8 ± 1.2</td>
<td>23.5 ± 1.3</td>
<td>0.8</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>LEUKOCYTES</td>
<td>-0.01 ± 1.56</td>
<td>-0.03 ± 1.70</td>
<td>&lt;0.1</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Blowfly pupae</td>
<td>2.5 ± 3.9</td>
<td>3.3 ± 5.0</td>
<td>0.6</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Fleas per nest</td>
<td>773.0 ± 422.8</td>
<td>418.8 ± 220.8</td>
<td>14.9</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

1Scores from a PC analysis of nestling wing, tarsus, head, and tail lengths.
2Scores from a PC analysis of nestling lymphocytes, monocytes, heterophils, eosinophils, basophils, and thrombocytes.
Fauth et al. (1991) removed greenery from starling nests once clutches were completed. Second, after clutches were complete, Gwinner et al. (2000) replaced natural nests with artificial greenery (herb) and grass (control) nests. Finally, Dawson (2004) added yarrow to tree swallow nests 4 or 5 days after clutch completion, and did not add more until nestlings were 6 days old. These studies may not have found effects because yarrow addition was not timed to be effective. First, starlings stop adding greenery after clutch completion and often as soon as the first egg is laid (Clark 1991), which suggests that if the chief benefit of this behaviour is to control ectoparasites, the key time to slow their population growth occurs early rather than late. Fauth et al. (1991) thus allowed volatiles to work for the normal interval before removing them, while Gwinner et al. (2000) and Dawson (2004) may have added too little too late. Second, starlings continuously replace greenery as it wilts, possibly because it stops giving off volatiles (Clark and Mason 1987), although it may be that only fresh greenery attracts mates (Brouwer and Komdeur 2004). If the former explanation holds, both Gwinner et al. (2000) and Dawson (2004) may not have used sufficient fresh greenery to prevent ectoparasite population growth. In addition, Gwinner et al. (2000) counted fleas in only 8 control and 7 greenery nests, so they had limited power to detect effects on this ectoparasite. Similarly, Dawson (2004) had only 5 control and 8 yarrow nests. Clearly, further evaluation of the efficacy of greenery is needed.

Experimental addition of yarrow had no significant effect on reproductive success or relative numbers of white blood cells, despite the dramatic reduction in fleas. We can only speculate as to why nestlings were larger in control nests because clutch sizes and number fledging did not differ. Possibly, as Saino et al. (1998) found with nesting barn swallows Hirundo rustica, faster feather growth occurred in the presence of ectoparasites, which may reflect adaptive, faster growth to escape infested nests. However, we found no relationship between flea numbers and nestling morphology. Another possibility is that yarrow is a stimulant to tree swallow nestlings that may have increased their metabolic rates and reduced resources available for growth. Alternatively, the difference in nestling size between control and yarrow treatments could represent a type I error.

It is tempting to ask why tree swallows have not evolved the behaviour of adding greenery to their nests, given its potential to reduce flea populations. If we assume that there is genetic or learning potential for this behaviour to evolve, we would need evidence that it would provide a significant benefit. However, there is little evidence that ectoparasites affect growth or survival of nestling tree swallows (Rogers et al. 1991, Thomas and Shutler 2001, Shutler et al. 2004). Møller and Erritzøe (1996) found that immune organs were consistently larger in hole-nesting versus open-nesting species, which they ascribed to greater likelihood of exposure to ectoparasites. Thus tree swallows nestlings may be born with immune systems that are already at peak capacity, and may be unable to respond to drug stimulants, preventing us from finding support for the drug hypothesis. Similarly, if yarrow does have negative consequences for nestling growth, this could outweigh the fitness benefits of reduced flea loads. Possibly, tree swallows nestlings are able to tolerate up to 2000 fleas per nest without lowered health. However, this cannot explain how swallow nestlings can tolerate so much blood loss without experiencing slower growth; the most likely explanation for this is that parents

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Control</th>
<th>Yarrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>0.19</td>
<td>0.22</td>
</tr>
<tr>
<td>Nestlings</td>
<td>0.32</td>
<td>0.03</td>
</tr>
<tr>
<td>Fledged</td>
<td>0.29</td>
<td>0.06</td>
</tr>
<tr>
<td>Proportion fledged</td>
<td>-0.09</td>
<td>0.55</td>
</tr>
<tr>
<td>SIZE1</td>
<td>-0.16</td>
<td>0.28</td>
</tr>
<tr>
<td>Mass</td>
<td>0.06</td>
<td>0.68</td>
</tr>
<tr>
<td>LEUKOCYTES2</td>
<td>0.08</td>
<td>0.98</td>
</tr>
</tbody>
</table>

1Scores from a PC analysis of nestling wing, tarsus, head, and tail lengths.
2Scores from a PC analysis of nestling lymphocytes, monocytes, heterophils, eosinophils, basophils, and thrombocytes.
compensate for nestling blood loss by increasing feeding rates (Johnson and Albrecht 1993, Tripet and Richner 1997). We have been unable to properly test this because experimental efforts to obtain similar flea numbers in a different population of tree swallows have failed (M. Hainstock and D. Shutler unpubl. data). The tradeoff between adding greenery versus a higher feeding rate may favour the latter, possibly because tree swallow bills are ineffective at tearing greenery from living vegetation, or because food is easy to come by when nestlings hatch.

There may be other reasons that birds bring greenery to their nests, such as to attract mates (mate choice hypothesis; Fauth et al. 1991, Eens et al. 1993, Gwinner 1997, Brouwer and Komdeur 2004). This hypothesis need not be mutually exclusive of the nest protection or drug hypotheses, but it clearly does not apply to tree swallows since they do not naturally add greenery to their nests. In any case, regardless of the current function(s) of adding greenery, it remains to be seen what the original function was for the behaviour.

There are other examples of organisms using substances with medicinal properties, but experimental data are often lacking to test whether this is adaptive (Clayton and Wolfe 1993). If the use of greenery by birds is an adaptation to reduce ectoparasites, three conditions must hold (Hart 1997). First, parasites that are negatively affected by greenery must, if left unchecked, affect host fitness. There is substantial evidence that ectoparasites can have these effects (Lehmann 1993, Richner and Heeb 1995, Møller et al. 1994). Second, greenery must decrease impacts of ectoparasites, which some studies have clearly demonstrated (Clark and Mason 1988, Clark 1991, this study), although the mechanisms by which the volatiles work remain to be elucidated (e.g., they could delay initial colonisation, inhibit oviposition, or affect feeding). Third, birds must preferentially select and discriminate greenery that has useful volatile compounds, which has also been shown (Clark and Mason 1987). However, there are clearly contradictory results regarding the efficacy of greenery in birds’ nests. Tree swallows offer significant potential as a species to use in future tests, but these tests need to more closely mimic the manner in which the animals pursue the strategies.

References


