



Green plants in starling nests: effects on nestlings

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European starlings, *Sturnus vulgaris*, intermingle fresh herbs, especially species rich in volatile compounds, with their otherwise dry nest material. In this field study we investigated whether these herbs reduce ectoparasites and thereby protect nestlings (the nest protection hypothesis). We also considered whether volatile compounds in herbs improve the condition of nestlings (the drug hypothesis). As measures of condition we used body mass, haematocrit levels and immunological parameters. We replaced 148 natural starling nests with artificial ones: half contained herbs and half (controls) contained grass. The ectoparasite loads (mites, lice, fleas) in herb and control nests were indistinguishable. However, nestlings in herb nests weighed more and had higher haematocrit levels at fledging than nestlings in control nests. Fledging success was similar in herb and control nests, but more yearlings from herb nests were identified in the colony the year after hatching. The response of the immune system when challenged with phytohaemagglutinin did not differ in nestlings from herb and control nests. Nestlings from herb nests had more basophils and fewer lymphocytes in their blood than those from control nests, while the eosinophil and heterophil counts did not differ. We conclude that herbs do not reduce the number of ectoparasites, but they improve the condition of nestlings, perhaps by stimulating elements of the immune system that help them to cope better with the harmful activities of ectoparasites.

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In the host–parasite arms race, some plants have evolved chemical weapons against herbivores and plant-injuring parasites. Herbs containing such secondary plant compounds are used not only in human medicine but also by some mammals, birds and insects for self-medication. Animals may chew plants, rub them into their fur, or make anting movements on plants containing biocidal substances (Clayton & Wolfe 1993; Hart 1997). Some bird species add fresh, aromatic plants to their nest material. This behaviour has been observed in purple martins, *Progne subis*, Indian house sparrows, *Passer domesticus*, South American monk parakeets, *Myiopsitta monachus*, American wood storks, *Mycteria americana*, blue tits, *Parus caeruleus*, South African mossies, *Passer melanurus*, buzzards, *Buteo buteo*, and other birds of prey, and European starlings, *Sturnus vulgaris* (Johnstone & Hardy 1962; Sengupta 1981; Wimberger 1984; Clark & Mason 1985; Bucher 1988; Rodgers et al. 1988; Roulin et al. 1997; Milton & Dean 1998; M. Lambrechts, personal communication).

Several hypotheses accounting for the use of green nest material have been proposed (for a review: see Clark 1991a, b).

The courtship hypothesis suggests that males use green plants to attract females (Kessel 1957; Feare 1984; Fauth et al. 1991). Male starlings carry herbs and tree leaves into their nests and also ornament them with flowers, lichens, bark, large feathers or artefacts such as ribbon, pieces of metal or plastic foil (Gwinner 1997). They frequently do this in the presence of females and mainly during pair formation. Males cease to incorporate greenery and other items when egg laying starts. From these findings, Gwinner (1997) concluded that green plants function as courtship tools.

An alternative, but not mutually exclusive, hypothesis, the nest protection hypothesis, suggests that compounds in green nest material control nest-dwelling ectoparasites (Widmann 1922; Wimberger 1984). A finding consistent with this is that birds breeding in holes, in which parasites are assumed to accumulate with repeated nest use, use green nest material more frequently than open-nesters, which use their nests only once (Clark & Mason 1985).

The plants that starling males most often show to females and deposit in their nests are rich in volatile compounds, which can have biocidal effects (Clark & Mason 1985; Gwinner 1997). Both in the laboratory and in field experiments in a starling colony in Pennsylvania, U.S.A., certain plants preferred by starlings as green nest material were found to impair the development of

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Table 1. Onset of laying, numbers of eggs, hatchlings, fledglings and nestlings that died, in herb and grass nests

	N herb/ N grass	Herb nest ($\bar{X} \pm \text{SE}$)	Grass nest ($\bar{X} \pm \text{SE}$)	Mann-Whitney <i>U</i> test	
				Z	P
Onset of laying	73/74	27.40 \pm 2.3	27.40 \pm 2.3	-0.54	0.59
Number of eggs	73/75	5.05 \pm 0.1	5.14 \pm 0.1	-1.10	0.27
Number of hatchlings	73/74	4.36 \pm 0.1	4.40 \pm 0.1	-0.46	0.64
Number of fledglings	68/68	3.63 \pm 0.1	3.84 \pm 0.1	-1.22	0.22
Number of nestlings that died	68/67	0.71 \pm 0.1	0.55 \pm 0.1	-1.06	0.59

northern fowl mites, *Ornithonyssus silviarum*, and bacteria (Clark & Mason 1988; Clark 1991b). In contrast, Fauth et al. (1991) found no effect of herbs on mites in nests in another American population of starlings.

In various bird species, hatching success, body mass and/or haematocrit and/or survival of nestlings is lowered in nests with high ectoparasite loads (Feare 1976; Hesse 1985; Fauth et al. 1991; Richner et al. 1993; Eeva et al. 1994; Dufva & Allander 1996). If herbs protect the nest from mites to improve the health of the nestlings, this should be reflected in their condition. However, no effect of plants on the body mass of nestlings has been detected in any study, although starling nestlings from nests in which mites were reduced by the presence of wild carrots, *Daucus carota*, had higher levels of haemoglobin than those from control nests (Clark & Mason 1988).

Research on the function of green plants in starling nests has so far been done only in the U.S.A. where the European starling has been introduced. Because the species of parasites and plants, and their interactions, may be different in Europe, where the plant-carrying behaviour evolved, different results might be obtained in a European starling colony. We investigated whether the herbs that male starlings preferentially weave into their nests reduce the numbers of ectoparasites and their deleterious effect on the condition of nestlings. As measures of condition, we used both body mass and haematological and immunological parameters.

METHODS

Treatment of Nests

We studied a nestbox colony close to Andechs, in southern Germany. Sixty nestboxes were spaced about 6 m apart along the margins of wet woodlands and meadows. The nestboxes had been swept irregularly up to 1994 but were not cleaned in subsequent years.

In 3 successive years (1995–1997) we exchanged 148 natural starling nests for artificial nests. These nests imitated an average starling nest in our colony, which weighed 100 g (range 65–130 g) and contained 40 g (range 0–350 g) of fresh, green plants. In 75 control nests (grass nests) 60 g of dry grass was supplemented with 40 g of fresh grass, which is rarely used as green nest material by starlings. Seventy-three experimental nests (herb nests) contained 60 g of dry grass mixed with 40 g of herbs of the kind preferred by male starlings as green nest

material (Gwinner 1997). Dry and fresh grass material belonged to the same species, *Brachypodium silvaticum*. We imitated the average green plant species composition and mass of different species by adding to each nest leaves of the following species: 10 g of goutweed, *Aegopodium podagraria*, 7 g of hogweed, *Heracleum sphondylium*, 7 g of elder, *Sambucus niger*, 7 g of cow parsley, *Anthriscus sylvestris*, 6 g of yarrow, *Achillea millefolium*, and 3 g of white willow, *Salix alba*. Male starlings normally stop incorporating greenery at the onset of laying. We exchanged the nests after completion of the clutches to minimize nest desertion by the females. Experimental and control nests were assigned such that the distribution of clutch sizes was 4 ± 1 in each group.

Onset of laying, numbers of eggs, hatchlings and fledglings and numbers of nestlings lost during the nestling period (=numbers of hatchlings minus numbers of fledglings) were statistically identical in experimental and control nests (Table 1).

Measurements of Nestlings and Immunological Parameters

Starling nestlings usually fledge at 20–23 days. We therefore refrained from checking the nests after day 19, to prevent them leaving the nestbox too early. Nestlings were weighed on days 1, 7, 14 and 18 ± 1 (=body mass at fledging) with a field balance to the nearest 0.1 g. All data listed below were obtained on day 18 ± 1 .

In all 3 years we took blood samples (9 μ l; <0.1% of fledgling mass) from the brachial vein. Haematocrit levels (ratio of red blood cells to total blood volume) were determined after centrifugation of the blood samples with a minicentrifuge Compur 1101 (Bayer Diagnostics, Munich, Germany).

In 1997 we also made immunological measurements. The thickness of the buffy coat, a layer of white blood cells on top of the red blood cells that separates during centrifugation, was measured with a binocular microscope on graph paper to the nearest 0.01 mm (Gustafsson et al. 1994). Leucocytes were counted from blood smears stained with 'Haema Schnellfaerbung' and numbers of basophils, eosinophils, lymphocytes and heterophils were determined per 100 leucocytes. We also tested T-cell-mediated immune responses of nestlings after injection with phytohaemagglutinin, PHA (Sigma-Aldrich Chemie, Deisenhofen, Germany), a mitogen inducing T-cell proliferation. The skin reacts with

swelling and inflammation. The thicker the swelling, the stronger the immune response. On the morning of day 17 we injected the left wing web of the nestlings with 0.4 mg of PHA dissolved in 0.04 ml of phosphate-buffered saline (PBS). The right wing web (control) was injected with 0.04 ml buffer only. Twenty-four hours later the thickness of the wing web was measured three times with digital callipers, always by the same person. We used the mean difference in thickness between the right and the left wing obtained from these three measurements as an estimate of the swelling response. The procedure of this test was in principle the same as described by Saino et al. (1997).

Estimation of Parasite Load

The most common ectoparasite in our starling colony was the red fowl mite, *Dermanyssus gallinae*, which produces three or four generations of mites during the 3-week nestling period of their hosts. All nymph stages and the adults feed on blood. The mites visit their hosts during the night for their blood meals and hide during the day (Sikes & Chamberlain 1954). In our nestbox colony mites assembled in clusters on the inner side of the lid and frame of the boxes. We scored mite infestation of the nestboxes on day 14, as follows: 0: no mites; 1: sporadic mites (no clusters); 2: clusters of mites covering up to 5 cm²; 3: clusters of mites covering more than 5 cm² of the nestbox surface. We reduced this score to two categories: 'low' (scores 0 and 1) and 'high' (scores 2 and 3).

Red spots ('scabs') on the abdomen of nestlings, resulting from the mites' blood meals (Feare 1984), were estimated on days 7 and 14: 0: no scabs; 1: less than 50%; 2: more than 50% of the abdomen covered with scabs.

Biting lice, Mallophaga, hide under the shoulders of the nestlings, when these are taken out of the nest. We estimated their numbers by checking the armpit at 7 and 14 days and recorded whether there were some (score 1) or none (score 0). In 1995 we collected the nests of the second brood, deep-froze them to kill the nest parasites, then shook the nests for 5 min in a sieve and determined the numbers of hen fleas, *Ceratophyllus gallinae*, from the siftings.

In 1995 we noticed that the differences in fledgling mass and haematocrit between nestlings from herb and grass nests were significant only when the mite load of the nests was high. In 1996 we therefore collected the initial content of all nestboxes in plastic bags, sieved it and returned it to both herb and grass nests to prevent reduction of parasite load.

Recording of Return Rate

We colour-ringed 147 nestlings from herb nests and 167 from grass nests and recorded all ringed birds that were observed in the colony the spring after hatching.

Statistics

Statistical analyses were carried out with the statistical packages SPSS version 8 and Genstat 5 release 4.1

(Genstat 5 1993). Depending on the data types, we used the following procedures. Our measure of association was Spearman's rho (for scores, counts and continuous variables that were not normally distributed). Differences in mite load and scabs between first and second broods in the same nestboxes were compared with the Wilcoxon signed-ranks test. For comprehensive analysis testing the simultaneous influence of several factors, we used multi-factorial ANOVAs (for normally distributed variables) and log-linear models (for scores). In log-linear modelling, we selected the best model by backwards elimination of factor combination. The impact of individual factors was assessed by *t* tests, Mann-Whitney *U* tests for non-normally distributed continuous variables and counts, and chi-square tests for categorical data and return rates. All sample sizes (*N*) refer to nests to avoid nonindependence of data. The data for each nest are means of nestlings per nest. Means are given \pm SE unless stated otherwise. All tests are two tailed.

Ethical Note

During 1995–1997, the 3 years of nest manipulation, 16, 13 and 17% of first-brood nests, respectively, were deserted during the incubation period. In 1994 when no nest exchange took place, 17% of the nests were deserted. The hatching success in our colony was 84.5%, similar to that reported for other sites in Europe and the U.S.A. (Feare 1984).

We measured the development of swelling of the wing web after PHA injection in six adult aviary starlings, using the procedure described above. The difference between the experimental and control wing webs was largest after 48 h ($\bar{X} \pm \text{SD} = 0.9 \pm 0.5$ mm) and decreased to almost 0 after 96 h (0.1 ± 0.1 mm). In chickens, *Gallus gallus domesticus*, the swelling after PHA injection disappears after 72–96 h (Goto et al. 1978), and in great tits, *Parus major*, after 96–120 h (M. Brinkhof, personal communication). In our field experiment we injected the nestlings at day 17 with PHA. Since starling nestlings usually fledge at 21 days of age, the swelling should have disappeared by then. All our PHA-treated nestlings fledged successfully. In 1998, the year after the PHA test, 7% of 120 nestlings ringed in 1997, returned to the colony. In 1996, when no PHA test was done, 115 nestlings were ringed and 7% of them returned to the colony the next year.

The birds were taken from the nests under licence from the Regierung von Oberbayern. For the blood samples and the PHA test no licences were required. Blood sampling had no adverse effects on the nestlings.

RESULTS

Nestling Condition and Ectoparasites

Red scabs on the nestlings, from both herb and grass nests, reflecting their burden of mites, and mite load of the nestboxes were positively correlated with each other ($r_s = 0.40$, $N = 86$, $P = 0.001$). Nestlings from nests with high mite loads ($N = 42$) had lower haematocrit levels than

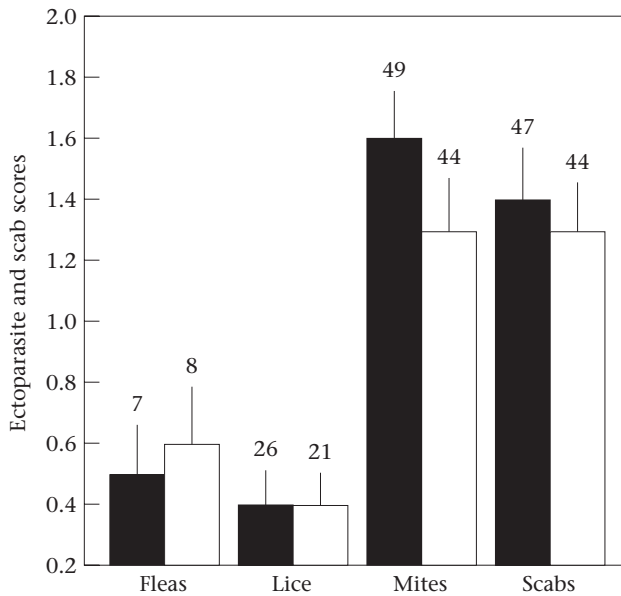


Figure 1. Ectoparasites and scabs on nestlings in herb (■) and grass (□) nests. Numbers of nests are given above the bars. For definition of scores see Methods.

those from nests with low mite loads ($N=49$; mite load high: $\bar{X} \pm \text{SE}=41.8 \pm 0.4$; mite load low: 43.5 ± 0.4 ; t test: $t_{88}=2.8$, $P=0.0001$). During the nestling period 0.8 ± 0.1 nestlings disappeared in nests with high mite loads and 0.5 ± 0.1 in nests with low mite loads (Mann–Whitney U test: $Z=1.7$, $N_1=50$, $N_2=44$, $P=0.08$). Fledglings did not differ in weight (mite load high: $\bar{X} \pm \text{SE}=68.9 \pm 0.67$; mite load low: 69.1 ± 0.55 ; t test: $t_{92}=0.17$, $P=0.86$).

Numbers of fleas, counted in 15 nests in 1995, correlated negatively with haematocrit levels of nestlings ($r_s = -0.7$, $P=0.006$), but showed no relation to body mass or numbers of nestlings lost during the nestling period ($r_s=0.03$, $P=0.92$; $r_s=0.08$, $P=0.77$).

Biting lice (Mallophaga) disturbed nestlings especially in their first week of life, when those carrying high lice loads ($N=33$) were lighter than those with low lice loads ($N=52$; lice load high: $\bar{X} \pm \text{SE}=40.8 \pm 0.9$ g; lice load low: 43.1 ± 0.7 g; t test: $t_{83}=1.99$, $P=0.05$). At fledging, these differences had disappeared (lice load high: $\bar{X} \pm \text{SE}=68.9 \pm 0.7$ g; lice load low: 69.05 ± 0.6 g; t test: $t_{83}=0.06$, $P=0.99$). This lack of difference at fledging was not related to nestling mortality. In nests with high lice loads 0.8 ± 0.1 nestlings disappeared and in nests with low lice loads 0.6 ± 0.17 nestlings disappeared (Mann–Whitney U test: $Z = -0.74$, $N_1=50$, $N_2=35$, $P=0.45$). From these relationships we concluded that mites, fleas and lice can adversely affect starling nestlings.

Nest Type and Ectoparasites

Herbs in the nest material had no detectable effects on the number of ectoparasites: the loads of mites, lice and fleas did not differ between herb and grass nests. Scores of red scabs on the abdomen were also similar between herb and grass nests (Fig. 1; fleas: $U=26.5$, $N_1=7$, $N_2=8$, $P=0.86$;

Mallophaga: $\chi^2_1=0.55$, $P=0.46$; scabs: $\chi^2_1=0.23$, $P=0.63$). We examined mite load more extensively in a log-linear model, since it also served as a factor for the subsequent analyses. By backward elimination, the log-linear analysis of mite load, year of study and nest type gave the best model when only the interaction between mite load and year was considered (likelihood ratio: $\chi^2_6=1.50$, $P=0.96$). Nest type made no appreciable contribution to the model ($\Delta\chi^2_1=0.26$, $P=0.61$).

Herbs can delay the development of mites, for instance by inhibiting their moult (Clark & Mason 1988). We therefore expected fewer mites in the nests and fewer red scabs on the nestlings later in the season, during the second brood, in nests that had been treated with herbs previously during the first brood. In June, during the second breeding period, all 21 inspected nestboxes contained more mites than in May during the first brood (Wilcoxon signed-ranks test: $Z = -3.31$, $N=21$, $P=0.009$). Nestlings also had more scabs during the second brood ($Z = -3.88$, $P=0.0001$). The increase in mites and scabs in the second-brood nests that had been provided with herbs ($N=12$) during the first brood was not different from that in nests that had contained no herbs ($N=9$) during the first brood (Mann–Whitney U test: mites: $U=39$, $P=0.66$; scabs: $U=118$, $P=0.98$).

Nest Type and Nestling Condition

Nestlings from herb nests were heavier and had higher haematocrit levels than those in grass nests; at 18 days, close to fledging, these differences were highly significant (Table 2, Fig. 2). Differences in mite load between years had no significant effect on body mass or on haematocrit; the better condition of nestlings in herb nests was thus not related to decreased mite infestation or to differences between years, but to the presence or absence of herbs (Table 2, Fig. 2).

The difference in body mass between nestlings from herb and grass nests was greater in nests where the mite load was high (t test: mite load high: $t_{52}=2.8$, $P=0.007$; mite load low: $t_{51}=1.8$, $P=0.07$; interaction nest type \times mite load: Table 2, Fig. 2a,b). The differences in haematocrit levels of nestlings from herb and grass nests tended to be higher in 1995, although not significantly so ($t_{42}=1.8$, $P=0.08$), than in 1996 ($t_{29}=1.2$, $P=0.25$) and 1997 ($t_{49}=-1.02$, $P=0.3$; interaction nest type \times years: Table 2).

Nest Type, Fledging Success and Return of Yearlings

The fledging success in herb and grass nests was the same (Table 1). However, the return rate of birds to the colony the year after hatching was higher for those from herb than grass nests: out of 314 colour-ringed nestlings (147 from herb nests, 167 from grass nests), 23 (7%) were identified in the colony the year after their birth. Of these 23 birds 15 (65%) were from herb nests and eight (35%) from grass nests ($\chi^2_1=4$, $P=0.05$). Five yearling males sang at nestboxes and defended them. Four of these males

Table 2. Condition of nestlings in relation to years, nest type and mite load

ANOVA	Body mass			Haematocrit			Lymphocytes			Basophils		
	df	F	P	df	F	P	df	F	P	df	F	P
Years	2	2.29	0.11	2	0.39	0.68						
Mite load	1	0.12	0.73	1	0.66	0.42	1	2.44	0.14	1	0.22	0.64
Nest type	1	8.02	0.006	1	9.64	0.003	1	8.6	0.009	1	7.80	0.01
Nest type×mite load	1	3.44	0.07	1	0.08	0.78	1	1.9	0.18	1	0.00	1.00
Nest type×years	2	2.39	0.10	2	5.35	0.006						
Years×mite load	2	0.46	0.63	2	2.89	0.06						
Error df	95			91			20			21		

Effect of years (1995–1997), mite load (high or low) and nest type (herb or grass nest) on body mass and haematocrit. For basophils and lymphocytes, counted in 1997, the effect of nest type and mite load was tested.

were from herb nests. Two of them, as well as two females, also both from herb nests, bred successfully in their first year.

Nest Type and the Immune System

Phytohaemagglutinin (PHA) test and buffy white layer

The swellings of the left wing web after injection of PHA and the right wing web (control) after injection of buffer did not differ in nestlings from herb and grass nests (0.997 ± 0.06 and 0.997 ± 0.07 mm; Mann–Whitney *U* test: $Z = -0.03$, $N_1=26$, $N_2=21$, $P=0.97$). There was, however, a relationship between mite load and the degree of swelling: nestlings from nests with low mite loads developed less swelling than those from nests with high mite loads (0.85 ± 0.04 and 1.4 ± 0.03 mm; Mann–Whitney *U* test: $Z = -2.60$, $N_1=34$, $N_2=12$, $P=0.009$).

White blood cells

The buffy white layer, a measure of white blood cells, did not differ in nestlings from herb and grass nests (0.41 ± 0.02 and 0.405 ± 0.02 mm; Mann–Whitney *U* test: $Z = -0.29$, $N_1=26$, $N_2=24$, $P=0.77$). There was also no difference in numbers of eosinophils and heterophils of nestlings from herb or grass nests (eosinophils of nestlings from herb/grass nests: $7.2 \pm 0.8/6.3 \pm 0.77$; Mann–Whitney *U* test: $U=71.0$, $N_1=N_2=13$, $P=0.5$; heterophils of nestlings from herb/grass nests: $20.3 \pm 1.7/18.2 \pm 1.6$; Mann–Whitney *U* test: $U=65.5$, $N_1=N_2=13$, $P=0.34$).

However, nestlings from herb nests had more basophils and fewer lymphocytes than those from grass nests; the mite load of the nestboxes showed no relationship to the numbers of basophils or lymphocytes (Table 2, Fig. 3).

DISCUSSION

Herbs and Ectoparasites

Ectoparasites can have negative effects on the fitness of their hosts. They may reduce growth and survival of nestlings (Richner et al. 1993; Oppliger et al. 1994; Merino & Potti 1995a), cause anaemia (Clark & Mason

1988; Fauth et al. 1991), influence clutch size (Richner & Heeb 1995), increase the metabolic rate of the nestlings (Møller et al. 1994) and affect dispersal (Brown & Brown 1992). Hosts can counteract these detrimental effects with increased preening and nest sanitation and by actively removing ectoparasites from the nestlings (Fraga 1984; Christe et al. 1996a) and/or increasing the feeding rate (Christe et al. 1996b). They can also mount adaptively induced defences against ectoparasites, which are reflected in better nestling conditions (Heeb et al. 1998). Some bird species, including starlings, use green plants as nest material, possibly to control nest parasites.

In our study, relatively high mite or flea loads, but not lice loads, were associated with relatively low haematocrit levels, indicating anaemia. Neither mite nor flea loads were related to the fledglings' mass but nestlings with high lice loads gained less mass during their first week of life.

We found no effect of our herb mixture on the mite, lice or flea infestations of the nestboxes. Some plants, such as wild carrots, have been shown to reduce the number of northern fowl mites in the nests of American starlings by preventing the mites from moulting (Clark & Mason 1988; Clark 1991b). If herbs had inhibited the development of mites in our study, we should have found fewer mites later in the season (i.e. during the second brood) in nestboxes that had been treated with herbs during the first brood. However, the mite loads and the red scabs on the nestlings' abdomens increased in a similar manner over the season in herb and grass nests. Hence, either our herbs did not impede the development of mites or the inhibition had no consequence for the host, because in the red fowl mite, unlike the northern fowl mite of the American study, nymph stages feed on blood as well (Sikes & Chamberlain 1954).

Two other studies also failed to reveal an effect of volatile plants on ectoparasites. The wood stork provides its nest with green plants such as cypress, red cedar, wax myrtle or pines. These preferred plants are rich in resins, and were, therefore, expected to repel ectoparasites; however, no effect on dermestid larvae, common ectoparasites of wood storks, was detected (Rodgers et al. 1988). Fauth et al. (1991) also found no effect of plant removal on mites and red scabs in starlings.

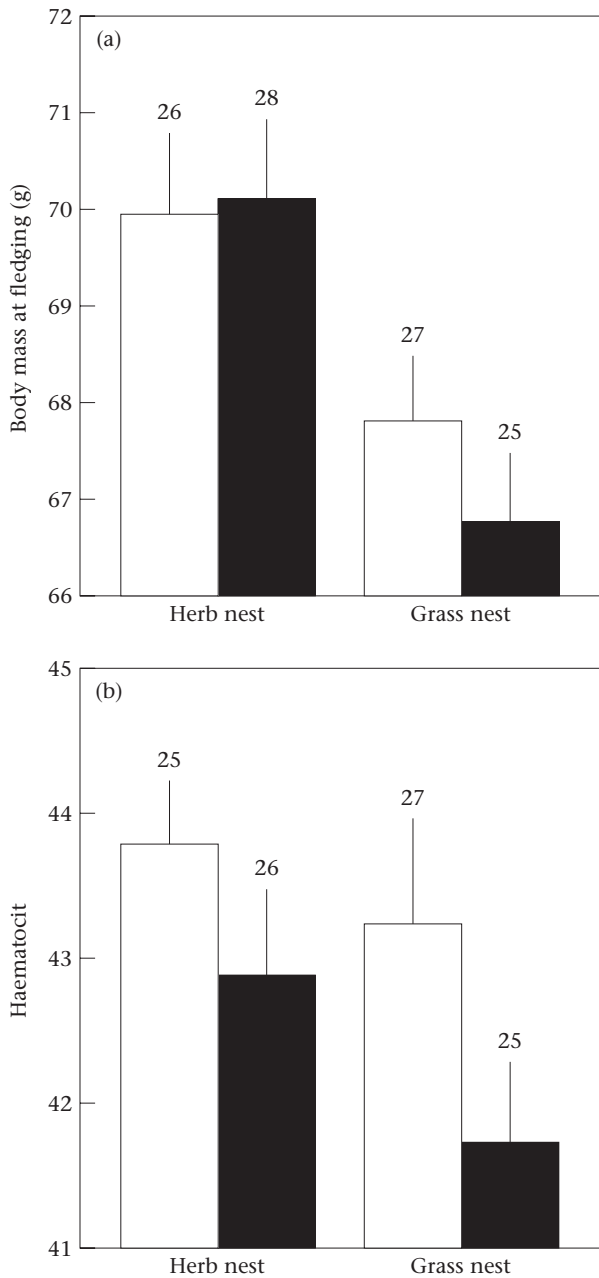


Figure 2. (a) Body mass and (b) haematocrit close to fledging of nestlings in herb and grass nests in relation to low (□) and high (■) mite loads. Means are given \pm SE. Numbers of nests are given above the bars.

Although various plants were available to the starlings at the breeding sites in southern Germany and Pennsylvania, those containing volatile compounds with potentially insecticidal properties were preferred by starlings at both sites. So, why was the northern fowl mite affected by wild carrots in the American study whereas the red fowl mite was unaffected by our plant mixture? The discrepancy may be caused by species-specific differences in plants or mites or their interaction. The plant composition at the two study sites clearly differed. For example, wild carrots, rarely found in starling nests in our

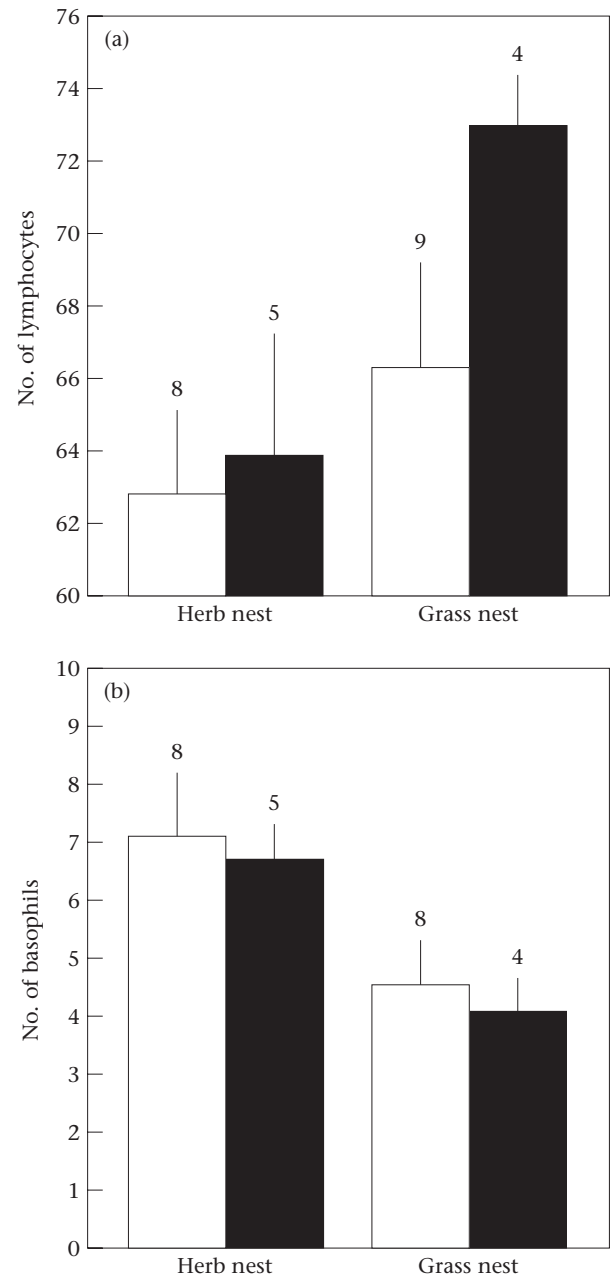


Figure 3. (a) Numbers of lymphocytes and (b) basophils per 100 leucocytes from nestlings in herb and grass nests in relation to low (□) and high (■) mite loads. Means are given \pm SE. Numbers of nests are given above the bars.

colony, were not in our experimental plant mixture. We therefore cannot compare our results with those of the American study directly. The reduction of mites by herbs in American nests could also reflect two different evolutionary stages of ectoparasite adaptation to host defence: American mites may not yet have evolved to counterbalance the defence strategies of a relatively new host. The experimental designs of the two studies also differed: Clark & Mason (1988) provided the nests with fresh plants several times during the incubation and nestling periods, while our procedure more closely

resembled what starlings do in nature in that no plants were introduced after the eggs had been laid.

In contrast to the American studies (Clark & Mason 1988; Fauth et al. 1991), we found an effect of green plants on body mass and haematocrit levels of nestlings. Nestlings from herb nests had higher body mass and haematocrit. These effects were especially obvious in nestlings from nests with high mite loads. Comparable associations were found in pied flycatchers, *Ficedula hypoleuca*: nestlings from low-quality territories weighed less than those from high-quality territories when the mite load was high, but not when it was low (Merino & Potti 1995b). Similarly, in house martins, *Delichon urbica*, bugs, *Oeciacus hirundinis*, had a greater detrimental effect on the nestlings when conditions were poor (De Lope et al. 1993). The absence of effect of herbs on body mass and survival of starling nestlings in the American studies could therefore have been a consequence of environmental conditions. Their data might have been collected under favourable conditions, that is, when cleaning and insecticide treatment of the nestboxes had kept the parasite load too low to harm the chicks.

Our data do not support the nest protection hypothesis in the sense that volatile plants repel or kill nest-dwelling arthropod ectoparasites. So far, we have not investigated their influence on other parasites or pathogens such as haematozoa, bacteria and fungi. Little is known about the prevalence of blood parasites in nestlings. In three studies on pied flycatchers, red-winged blackbirds, *Agelaius phoeniceus*, and kestrels, *Falco sparverius*, no haematozoa were found in nestlings (Bennett et al. 1974; Apanius & Kirkpatrick 1988; Weatherhead & Bennett 1991). In one study, 21% of pied flycatcher nestlings from central Spain were infected with trypanosomes (Merino & Potti 1995b). So far, starlings have never been found to be infected with trypanosomes (Gylstorff & Grimm 1987), and their occurrence in nestlings is thus unlikely. Coccidia were present in 1.2% of various ground finch species of the Galapagos (Mcquistion 1990), and in 14% of Canadian puffin, *Fratercula arctica*, nestlings (Leighton & Gajadhar 1986). In 50 blood smears of starling nestlings from 25 nests, no blood parasites were seen (H. Gwinner, unpublished data). These few studies suggest that haematozoa, coccidia and bacteria occur only rarely in nestlings, and that their effect on nestlings may therefore be negligible.

There is a further explanation, consistent with the nest protection hypothesis, for the effect of herbs on the nestlings' condition. Increased corticosterone levels in chickens inhibit blood sucking by the red fowl mite (Hall & Gross 1975 cited in Clark 1991b). Some plants, such as the Echinaceae, are known to stimulate corticosterone secretion (Steinegger & Haensel 1988). It is thus conceivable that herbs could affect the feeding behaviour of mites.

Herbs and Local Recruitment

More starlings from herb nests than from grass nests returned to their native colony in the year after their birth and some of them, unusually for yearlings, even bred there. This difference may have any of several causes.

(1) Birds reared in herb nests may have survived better. (2) Herb treatment may have advanced the age of reproductive maturity. (3) Given that starlings can discriminate odours (Clark & Mason 1987), birds reared in herb nests may have found the place of their birth more easily by recognizing the smell of the herbs at the site. That starlings are capable of finding their breeding colony by smell was shown by Wallraff et al. (1995), who found in the same starling colony that fewer starlings returned to their breeding sites when they had been deprived of the sense of smell.

Herbs and the Immune System

Hypersensitivity to the plant-derived protein phytohaemagglutinin (PHA), as measured by the amount of swelling of the wing web after an injection with PHA, was not influenced by providing the nests with green material. However, nestlings from nests with high mite loads reacted with larger swellings to the challenge of the immune system, which might have been sensitized by factors introduced when mites suck blood, as shown for pigeons, *Columba livia*, and a species of tick (Dusbabek et al. 1988).

Basophils occurred in greater, and lymphocytes in smaller, numbers in the blood of nestlings from herb nests. In general, basophils synthesize and secrete mediators, which control the development of immune responses. They are more frequently found in birds than in mammals, where they respond to allergens, but also play a role in immunity against parasites (Roitt et al. 1996). In birds, basophils increase the capacity to cope with stress induced by climatic factors or by malnutrition; they also play a role during inflammation, where they perform phagocytosis (Maxwell et al. 1990, 1992; Sundaresan et al. 1990; Savory et al. 1993; Maxwell & Robertson 1995).

We do not know how plant compounds stimulate the immune system of birds. In human medicine, extracts of certain plants (*Echinacea*, *Bryonia*, *Viscum*, *Baptisia*) are used as 'immunostimulants', which are thought to activate effector mechanisms of the defence system. In humans, plant extracts stimulate phagocytosis of macrophages, and increase monokines and natural killer cells (Steinegger & Hänsel 1988).

On the basis of these known interactions, we hypothesize that compounds in the herbs tested in our study stimulated elements of the immune system, enabling the nestlings to cope better or faster with stress in general and ectoparasites in particular. Herbs did not reduce the number of ectoparasites, but possibly helped nestlings to withstand their detrimental influences better.

The Drug Hypothesis

The better condition of nestlings in herb nests was reflected in their higher body mass and haematocrit levels and, perhaps, a better resistance against parasites. In addition it seems that their local recruitment, an important component of fitness, was better. As one possible

explanation, we therefore suggest the 'drug hypothesis', which proposes a direct positive effect of herbs on the health of nestlings.

Alternative Hypotheses

In our experiment we tested the effect of fresh herbs, preferred as nest greenery by starlings, against the effect of fresh grass, which was rarely used as green nest material by starlings (Gwinner 1997). One could argue that our treatment did not create better conditions in herb nests but worse conditions in grass ones, because of harmful effects of grass. We consider this explanation unlikely for two reasons. (1) The grass species we used was the same the starlings used as dry nest material. (2) If herbs had no positive effect one would expect them not to be chosen selectively but rather to be collected according to their frequency at the nest sites. We found, however, in the field (Gwinner 1997) and in choice experiments in the aviary (Lambert 1997), that starling males prefer volatile plants over nonvolatile plants (including grass).

Possibly, parental behaviour was affected by our treatment. Because they expect a lot of herbs in the nest of a high-quality male, females may invest less in nestlings in grass nests (of low-quality males?) and feed them less. Alternatively, females that had incubated in herb nests might have been in better condition and therefore fed their nestlings better. Some unpublished data contradict both these ideas. We observed feeding activity for 90 min at seven grass and nine herb nests each with four nestlings, and found that mothers from herb/grass nests fed 3/3.3 times, and fathers from herb/grass nests 1/0.9 times, per 10 min (Mann-Whitney U test: $P < 0.5$; H. Gwinner, unpublished data).

Other possibilities are that volatiles from aromatic herbs act as appetizers (Steinegger & Haensel 1988) so that nestlings in herb nests fed more and therefore became heavier or that volatile plants provide better insulation (Clark 1991b). Consistent with the latter idea is our observation that differences in body mass of nestlings in herb and grass nests were greatest in 1996, when heavy rains and low temperatures predominated during the early nestling period.

The Courtship Hypothesis

Green plants may be involved in two aspects of the starlings' breeding biology. First, they may serve as courtship tools to attract females and, second, they may have a favourable effect on the chicks' condition. Male courtship displays are often interpreted as signals enabling the mate-choosing female to evaluate her prospective mate's state of health or the 'quality of his genes'. Movements originally used during preening, feeding or nest building can occur in a ritualized form during courtship. By adding greenery to the nests, starling males include an element of nest-building behaviour in their courtship behaviour. After having shown the plants to the female, males weave them into the nests for the benefit of their young.

Polygynous starling males, which defend several nest-boxes, carry more green plants into their second or third nests than monogynous males in their single nest and so may signal their good qualities to females (Gwinner 1997). Polygynous males help their additional females less with feeding the young (Pinxten & Eens 1994; Smith 1995), but perhaps compensate for this deficiency with a greater herb gift to their offspring. Displaying plants during courtship could therefore be interpreted as a signal simultaneously transmitting two kinds of information to a female: first, that her mate is of good quality; and second, that her offspring will be in good condition.

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