

CHEMORECEPTION AND THE SELECTION OF GREEN PLANTS AS NEST
FUMIGANTS BY STARLINGS

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INTRODUCTION

Although once controversial, it is now well-established that many avian species use volatile chemical cues. This is the case even though birds lack a vomeronasal organ (Portmann, 1961). Chemoreception is not restricted to those species with anatomically well-developed olfactory and/or trigeminal systems. Songbirds and other birds (e.g., pigeons) with relatively undeveloped olfactory anatomy nonetheless use volatile cues for a variety of purposes, including feeding (Archer and Glen, 1969; Stager, 1967; Snyder and Peterson, 1979; Wenzel, 1968), homing (Benvenuti et al., 1973; Grubb, 1974; Papi et al., 1973; Wallraff, 1980), and, possibly, communication (Frings and Jumber, 1954). Because comprehensive reviews of these topics are available (e.g., Kare and Mason, 1985; Wenzel, 1973, 1980), we choose not to review them here. Instead, we will suggest that some birds may use chemical cues for a quite different purpose. We will present natural history, and behavioral, chemical, and electrophysiological data consistent with the hypothesis that birds that reuse nest sites use green plants as nest fumigants, and that they choose plants for incorporation into the nest on the basis of chemical cues. This use of green vegetation represents the first documented case of an air-breathing vertebrate using the chemical defenses of another organism for its own purposes.

NATURAL HISTORY

Many species of birds incorporate fresh green vegetation into their nests. Several hypotheses have been advanced to account for this behavior. Early naturalists ascribed to birds an esthetic sensibility for seeking brightly colored flowers (Welty, 1975). More recent interpretations have suggested selective benefits, including a form of sexual selection analogous to the courtship-related activities of bower birds (Collias and Collias, 1984). Fresh vegetation also might alter the insulative properties of nests (Kern, 1984), or maintain a high vapor pressure within the nest environment, thereby preventing desiccation of eggs (Mertens, 1977). In addition, nest material may closely match surrounding vegetation, concealing the nest from predators visually searching for prey (Lack, 1954; Skutch, 1967). Finally, chemicals contained in fresh vegetation may act as repellents, contact toxicants, or fumigants, effective at controlling parasite and/or pathogen

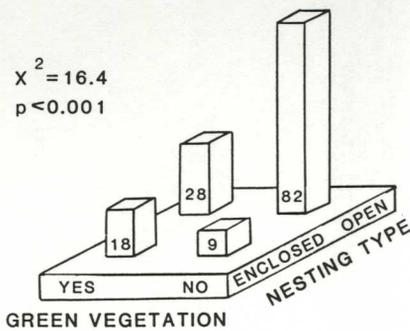


Fig. 1. The frequency distribution of nests of 137 eastern North American passerines as a function of nesting type (open versus closed), and whether or not the species incorporates fresh green vegetation into its nest matrix (Clark and Mason, 1985).

populations (Meyers, 1922, Johnston and Hardy, 1962; Sengupta, 1981; Wimmerger, 1984; Clark and Mason, 1985).

The long term use of nest sites during a single breeding effort, or over several breeding efforts within a single season, increases the risk of incurring large parasite or pathogen loads (Stoner, 1936; Wasylik, 1971; Powlesland, 1977). Also, viruses, fungi and bacteria can lie dormant in nest debris and feces for several months, withstand freezing temperatures (Davies et al., 1971) and thereby increase the risk of infection to birds breeding at historically active sites. The inability to cope with parasite or pathogen infestation has led to nest desertion, egg spoilage, and juvenile mortality among several species (Neff, 1945; Szybalski, 1950; Florian and Trussell, 1957; Davies et al., 1971; Oliphant et al., 1976; Feare, 1976; Duffy, 1983) and is known to decrease vigor of nestlings which may diminish their future survivorship probabilities (Moss and Camin, 1971; Powlesland, 1977; Gold and Dahlsten, 1983; Whiteman and Bickford, 1983).

The case for plant compounds acting as a chemical defense against their own herbivores and pathogens is well established (e.g., Rosenthal and Janzen, 1979). We set out to test the hypothesis that some species of birds use attributes of fresh green vegetation to increase their probability of reproductive success. As an initial step, we reasoned that if fresh plants contain chemical compounds that act as contact toxicants or natural fumigants to the birds' ecto-parasites or pathogens, then species more likely to nest in previously used sites should be more prone to include fresh plant material into their nests. A review of the nesting behavior of 137 passerine species breeding in eastern North America was consistent with this hypothesis. Passerines nesting in enclosed spaces (e.g., secondary cavity nesters or species using crevices) were more likely to incorporate green plants into their nests, while passerines that infrequently reused nest sites and nested in open, cup-like nests were less likely to incorporate green vegetation into their nests (Fig. 1). Notable exceptions occurred. Some secondary cavity nesting species such as Nuthatches (e.g., *Sitta canadensis* and *S. carolinensis*) did not place fresh plant material into their nests. These species did, however, place pine pitch around their entrance holes, or squeeze and rub insects on the surface of the cavity (Bent, 1965c).

SPECIFIC QUESTIONS

We addressed specific questions regarding the use of nest material by a single species, the European Starling (*Sturnus vulgaris*). Four questions were asked. First, did Starlings select particular species of plants from the available vegetation for incorporation into their nests? If so, then were plants selected by Starlings more effective at controlling ectoparasites or bacterial pathogens than a random subset of vegetation available in the habitat? Third, were the chemical profiles of volatiles found in plants used by Starlings different from profiles of a random subset of vegetation available in the habitat? Finally, we asked whether Starlings could discriminate among plants on the basis of chemosensory information alone.

Study Site and Breeding Behavior

The study site was a Starling nesting colony of about 100 nest boxes located at the Stroud Water Research Center of the Philadelphia Academy of Natural Sciences, Avondale, Pennsylvania. The habitat lies within the White Clay Creek watershed and is characterized by a patchwork of old fields (maintained via semi-annual mowing), pastureland, and secondary growth woodland.

Detailed accounts of Starling nesting behavior have been provided by several authors (e.g., Dunnet, 1955; Feare, 1984; Kessel, 1959). Observation of banded birds at the Stroud Water Research Center revealed the following patterns. Males intermittently visit the nesting area from August to March, show interest in cavity sites beginning in December or January, and throughout February, spend increasing amounts of time during the morning in or around one or two nest boxes. At this time, they may begin to clean out the previous season's nest material and debris. By March, males from the local population are well established at a cavity site, vigorously singing at the nest box during the early morning, and defending the nest site against newly arrived birds. Males also begin to place bits of fresh vegetation in the nest cavity. These bits are the tips of new growth and range between 1 and 3 cm in length. The behavior of placing only fresh vegetation in nest cavities lasts throughout March and into the first week of April. The number of species of plants found in the cavity increases with the availability of vegetation.

Males initiate nest construction in early April, and are aided subsequently by females. Nests consist of bulky masses of dried grasses of the previous year's growth. Woven into the matrix of dried grasses are bits of fresh green plant material. Bits of fresh vegetation are added continually to the nests until the eggs hatch, and then the behavior falls off dramatically.

(1) Do Starlings Select Particular Plants for Incorporation Into Their Nests?

Nest material. We checked nests for green vegetation every three days, from the initial stage of nest building to the hatching of young. We removed the nests from the boxes, picked apart the matrix by hand, and removed all green vegetation. The green vegetation was labeled and pressed for later identification. Thirty-eight herbaceous species were recorded, although a small fraction of green vegetation (< 5%) could not be identified to species. Dried nest matrix was reassembled, and placed back into the nest box. No birds abandoned their nests as a result of this manipulation.

Vegetation survey. During peak nest-building in the last week of April and the first week of May 1984, we sampled the green herbaceous vegetation

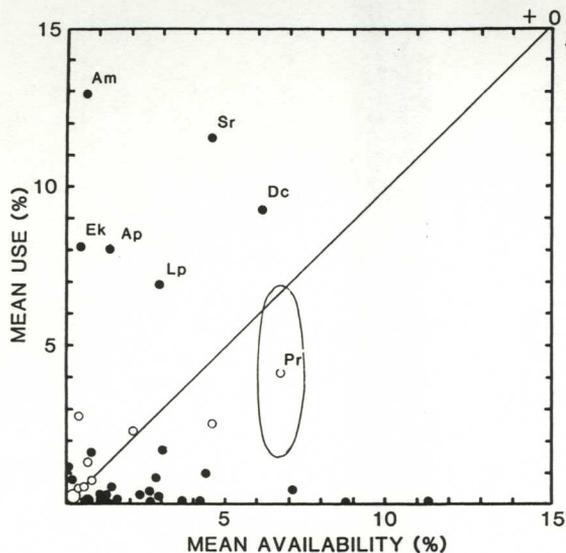


Fig. 2. Mean % use in nests versus mean % availability in habitat for 66 herbaceous species. The diagonal line represents a hypothetical selection scheme by Starlings, in which plants are selected in proportion to their abundance. Species selected in proportion to their abundance are represented by open circles and are classified as those species whose sampling errors along either axis intersected the diagonal line (e.g., Pr.) (Clark and Mason, 1985). Solid circles above the diagonal represent species selected in excess of their proportional abundance. Solid circles below the diagonal represent species selected in numbers less than proportional abundance. The large open circle is for 22 overlapping points, while the large solid circle is for 5 overlapping points. Species abbreviations are Am: Achillea millefolium; Sr: Solidago rugosa; Dc: Daucus carota; Ek: Erigeron sp.; Ap: Agrimonia parafiora; Lp: Lamium purpureum; Pr: Polemonium reptans.

in plots randomly placed along transects throughout the breeding colony. A total of 66.5 m² plots were sampled along 24 transects. This sampling effort was adequate, because starlings usually fly less than 50 m to choose material for their nests (Kessel, 1959; Bent, 1965).

Eighty-four herbaceous species were collected (samples of each plant were frozen for later use). Because the asymptotic number of plant species found in the habitat was approached after sampling 30 plots, we concluded that our sampling effort had in fact provided an adequate description of the available vegetation. Starlings selected nine species of plants in numbers greater than their proportional availability, 34 in proportion to their availability, and 23 species in numbers less than their availability from the habitat (Fig. 2). Such a pattern of vegetation use was not expected from a chance utilization of available vegetation (one-tailed Kolomogorov-Smirnoff test, $p < 0.005$). Too few plants were classified as being preferred by Starlings. For subsequent experiments, we used the six most frequently preferred species and six randomly selected species that Starlings either avoided or selected in proportion to their availability (Table 1).

Table 1. Plants chosen in excess of their availability ("preferred") versus plants chosen either in proportion to or below availability ("other").

Preferred Plants		Other Plants	
Scientific Name	Common Name	Scientific Name	Common Name
<u>Agrimonia</u> <u>paraflorea</u>	Agrimony	<u>Potentilla</u> <u>recta</u>	Cinquefoil
<u>Daucus</u> <u>carota</u>	Wild carrot	<u>Brassica</u> <u>nigra</u>	Black Mustard
<u>Erigeron</u> sp.	Fleabane	<u>Polemonium</u> <u>reptans</u>	Greek Valerian
<u>Achillea</u> <u>millefolium</u>	Yarrow	<u>Claytonia</u> <u>virginica</u>	Spring Beauty
<u>Lamium</u> <u>purpureum</u>	Red-dead Nettle	<u>Conium</u> <u>maculata</u>	Poison Hemlock
<u>Solidago</u> <u>rugosa</u>	Rough Goldenrod	<u>Viola</u> sp.	Violet

(2) Are Plants Selected by Starlings More Effective at Controlling Ecto-parasites and Bacterial Pathogens than a Random Subset of Other Available Vegetation?

Ecto-parasites. Eggs of the louse, Menacanthus sp., and adult fowl mites, Ornithonyssus sylviarum were collected from adult birds and nests, respectively. This choice of ecto-parasite species was based on their potential for negative impact on nestling growth and survivorship (Moss and Camin, 1971; Powlesland, 1976). Logistical constraints determined which instar of each ecto-parasite was used.

Eggs of the louse were found attached to the base of feathers on the heads and necks of adult birds (Boyd, 1951). These eggs were removed and placed in covered, humidified petri dishes. Adult fowl mites were collected from infested nest boxes by placing a strip of paper amidst a swarm of mites, allowing the mites to board the paper, then depositing the mites and paper into a vial. Upon return to the laboratory, the mites were transferred to covered, humidified petri dishes.

Experimental treatments consisted of placing 0.2 g of dried plant material into randomly selected petri dishes. These dishes, and control dishes containing eggs or mites but no plant sample, were incubated at $30 \pm 1^\circ\text{C}$, which is within the range of temperature of feathers on live birds (Clark, pers. obs.). The viability of louse eggs (whether or not they hatched) and mortality among mites was checked every other day for eight days. At no time was contact permitted between plant samples and Menacanthus eggs during experiments.

Bacterial pathogens. Four standard laboratory strains of bacteria (Streptococcus aurealis, Staphylococcus epidermis, Pseudomonas aeruginosa, and Escherichia coli) were chosen on the basis of literature pertaining to

Table 2. The effectiveness of plant material at inhibiting bacterial growth on a nutrient medium: (++) represents strongly bactericidal; (+) moderate effectiveness; blank for ineffective plants. The asterisk denotes plant species used preferentially by Starlings. Codes are Se: Staphylococcus epidermis; Pa: Pseudomonas aeruginosa; Ec: Escherichia coli; Sa: Streptococcus aurelia; and NB from nests and nest boxes. Unk. 21 is an unidentified plant species.

Plant Species	Se	Pa	Ec	Sa	NB
<u>Solidago rugosa</u> *				+	+
<u>Solidago ulmiflora</u>					
<u>Conium maculatum</u>					
<u>Agrimonia paraflora</u> *	++			++	++
<u>Achillea millefolium</u> *					+
<u>Polemonium reptans</u>					
<u>Glechoma hederacea</u>					
<u>Geum virginianum</u>					
<u>Geum canadense</u>				+	
<u>Taraxacum officinale</u>					
<u>Lonicera japonica</u>				+	
<u>Daucus carota</u> *		+		+	
<u>Alliaria officinalis</u>					
<u>Senecio obvatus</u>					+
Unk. 21					
<u>Viola</u> sp.					
<u>Lamium purpureum</u> *					

the pathogenicity of these strains to birds or eggs (Davies et al., 1971). In addition, unidentified wild strains of bacteria were collected from nest boxes by swabbing the nest and nest cavity with sterilized cotton swabs dipped in sterilized distilled water. Each contaminated swab was carried back to the laboratory in a sterilized tube. Sterilized nutrient medium was inoculated with the standard strains or wild strains, and then each experimental plate was incubated in the presence of a 1 cm² piece of sterilized leaf material. There were three replications for each plant tested and a control (i.e., a plate inoculated with bacteria but without leaf material). Our presumption was that any bacteriocidal compounds present in the leaves would diffuse into the nutrient medium and inhibit bacterial growth. Plants were scored as strongly bacteriocidal if there was a bacteria free zone around leaf pieces greater than 1 cm. Plants were scored as moderately effective if a bacteria free zone was apparent, but less than 1 cm. Plants were scored as ineffective if no apparent inhibition of bacterial growth was observed.

Results. Plants preferred by Starlings retarded the hatching of Menacanthus sp. (one-tailed $t = 3.39$, $p < 0.005$) whereas other randomly chosen plants did not. Also, plants effective at controlling the growth of pure strains of S. aurealis, S. epidermis, P. aeruginosa, and wild bacteria obtained from nest cavities were more frequently used by Starlings (one-tailed $t = 2.05$, $p < 0.05$). No plants were effective against E. coli (Table 2). Also, no plants were effective against adult fowl mites (O. sylviarum). In fact, after three days of exposure to the plants, no mites had died in either group, and after five days of exposure, only a few mites from any group had died.

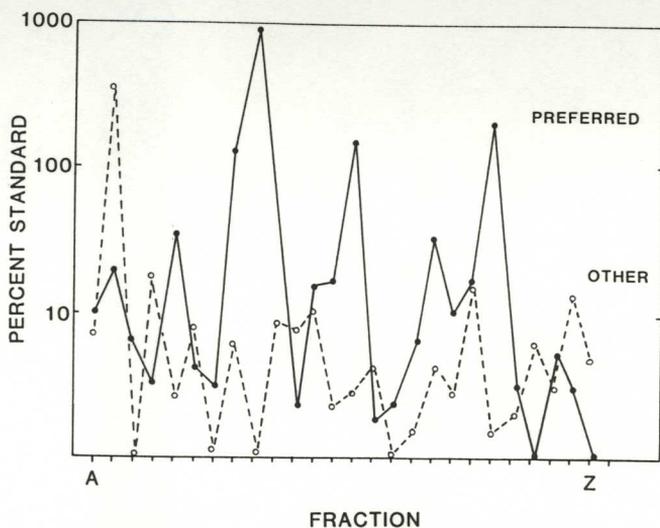


Fig. 3. A profile for the mean concentrations of volatile chemicals in preferred versus other plants. Each fraction represents either a single compound or a group of compounds based on relative retention times from a gas chromatograph.

(3) Are Chemical Profiles of Volatiles Found in Plants Used by Starlings Different From Profiles of a Random Subset of Vegetation Available in the Habitat?

The phytochemical analysis of plant volatiles was carried out in collaboration with Dr. John Labows, an analytical chemist at the Monell Center. Only large scale patterns of similarity among plants are reported here. Plant samples were thawed and blotted dry. Five grams of leaf tissue were placed in a 250 ml flask, and volatiles were collected from the headspace of the flask. Gas chromatographic and mass spectrographic techniques were used to identify potentially active fractions of plant volatiles and the identity of those fractions (Clark and Mason, in press). Plants preferred by Starlings contained significantly higher concentrations of volatile compounds (Wilcoxin matched-pairs test, $z = -1.73$, $p < 0.042$; Fig. 3)., although a cluster analysis did not reveal any other clear-cut dichotomy between preferred and other plants (Clark and Mason, 1985). GC/MS analyses indicated that the volatiles contained within preferred plants were mostly mono- and sesquiterpenes, such as myrcene, α - and β -pinene, cymene, ocimene, α phellandrene, α terpineol, Δ^3 -carene, sabinene, α terpinolene and limonene. At least one of the preferred plants, *Solidago rugosa*, contained the sesquiterpenes 2-bornyl acetate and farnesol. These compounds have been implicated as potent insect juvenile hormone analogs (Rosenthal and Janzen, 1979). Such compounds generally suppress molting in arthropods. Any such action of compounds on ecto-parasites would delay the timing of terminal instars, and hence curtail the reproductive capacity of the parasites. As a consequence, the rate of population growth by parasites, and by implication, their adverse effect on nestling birds (Powlesland, 1978), would be reduced. Detailed analysis of the chemical composition of the plants is forthcoming.

(4) Can Starlings Discriminate Among Plant Species on the Basis of Volatile Chemical Characteristics?

Obvious sensory cues that the birds could use for discrimination among plants include visual characteristics. For example, passerines can make fine visual discriminations among leaf shapes (Real et al., 1984). Other possible cue sources mediating discrimination might be volatile or non-volatile chemical cues. Many of the used plant species are aromatic to human observers. Because we had already collected evidence suggesting that Starlings possess acute chemosensory abilities (e.g., Mason et al., 1985; Mason and Silver, 1983), we designed a series of electrophysiological and behavioral experiments to test the possibility that Starlings can discriminate among plants on the basis of some volatile cue.

Electrophysiology

Integrated multiunit responses were obtained from five adult Starlings. The birds were food deprived for approximately 12 hours before the experiment, anesthetized with urethane (4.6 ml/kg, ip), and placed into a head-holder. The feathers, skin, and bone between the eyes was removed. A small Teflon cap was fitted to the cleared area and fixed in place with dental cement. This cap provided a reservoir for mineral oil, which was pipetted into the cap to prevent desiccation. The olfactory nerves under the bony orbital walls were exposed, and electrical activity was recorded from the left olfactory nerve by dissecting a small twig free, and placing it on a pair of platinum-iridium wire electrodes. The preparation was grounded through the head-holder apparatus. The platinum-iridium hook electrodes were connected to the high impedance probe of an AC coupled preamplifier. The amplified neural activity was monitored with a storage oscilloscope and an audio analyzer, and stored on magnetic tape. Multiunit activity was summed using a short-time averaging circuit with a time constant of 0.5-1.0 sec (Kiyohara and Tucker, 1978), and displayed on a pen recorder.

The magnitudes of the neural responses were measured in arbitrary units from the baseline to the peak of the phasic response. Responses were reported as a percentage of the response to a standard stimulus, air saturated with n-butanol at 23°C.

Birds were tested for integrated neural activity in response to eight stimuli: the arbitrary standard (n-butanol), a blank that consisted of ambient air passed through the delivery apparatus, and volatiles from six plant species: Agrimona parafiora, Daucus carota, Achillea millefolium, Aster prenanthoides, Aster praealtus, and Solidago rugosa. Volatiles from 10 g of each plant were allowed to accumulate in 125 ml flasks for 15 min at 23°C. Fifty cc of headspace from each flask was then withdrawn by syringe, and injected into the left naris of each bird at a rate of 10 cc/sec. Five min interstimulus intervals were used, and as many series of the eight stimuli as possible were presented to each bird at 20 min intervals.

Results. Without exception, n-butanol, and volatiles from all the plant species elicited vigorous responses from the Starlings' olfactory nerves (Fig. 4). Room air also elicited responses, although to a considerably lesser extent. We took these results as evidence that Starlings have the olfactory capacity to detect volatiles from both preferred and unused plants. Experiments were then designed to determine whether Starlings could behaviorally detect and discriminate among plant odors.

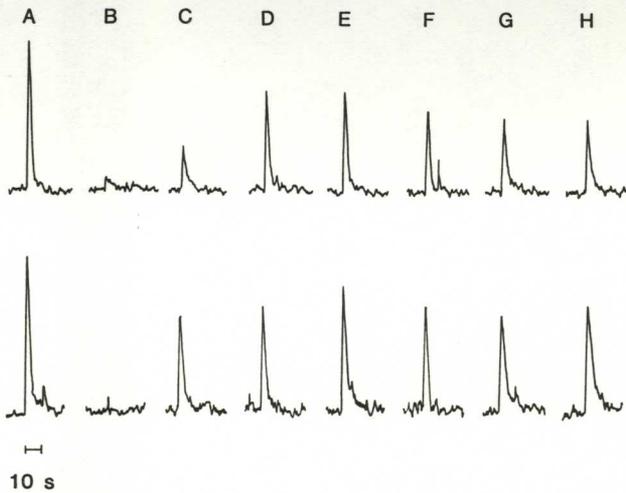


Fig. 4. Integrated multineuronal responses from the olfactory nerves of two birds. Stimuli were: (a) n-butanol, (b) room air, (c) Agrimonia parafiora, (d) Aster prenanthoides, (e) Solidago ulmiflora, (f) Solidago rugosa, (g) Aster praealtus, (h) Achillea millefolium.

Behavior

Eight independent experiments (Table 3) were performed to determine whether Starlings could detect and discriminate among volatiles from plants. For each investigation, birds were individually housed and visually isolated eight days prior to training. After four days, the birds were adapted to a deprivation regime in which they were food deprived during the first

Table 3. Plant species used for adaptation and two-choice behavioral tests in eight independent experiments.

	<u>Adaptation</u>	<u>Two-Choice Tests</u>
1.	<u>Solidago canadense</u>	<u>Solidago rugosa</u> vs. <u>Allaria officinalis</u>
2.	<u>Aster</u> sp.	<u>Solidago rugosa</u> vs. <u>Geum canadense</u>
3.	<u>Alliaria officinalis</u>	<u>Glechoma hederacea</u> vs. <u>Ranunculus</u> sp.
4.	<u>Ranunculus</u> sp.	<u>Polemonium reptans</u> vs. <u>Erigeron</u> sp.
5.	<u>Allaria</u> sp.	<u>Plantago major</u> vs. <u>Lamium purpureum</u>
6.	<u>Potentilla recta</u>	<u>Achillea millefolium</u> vs. <u>Brassica nigra</u>
7.	<u>Veronica</u> sp.	<u>Agrimonia</u> sp. vs. <u>Potentilla recta</u>
8.	<u>Solidago canadense</u>	<u>Barbarea vulgaris</u> vs. <u>Daucus carota</u>

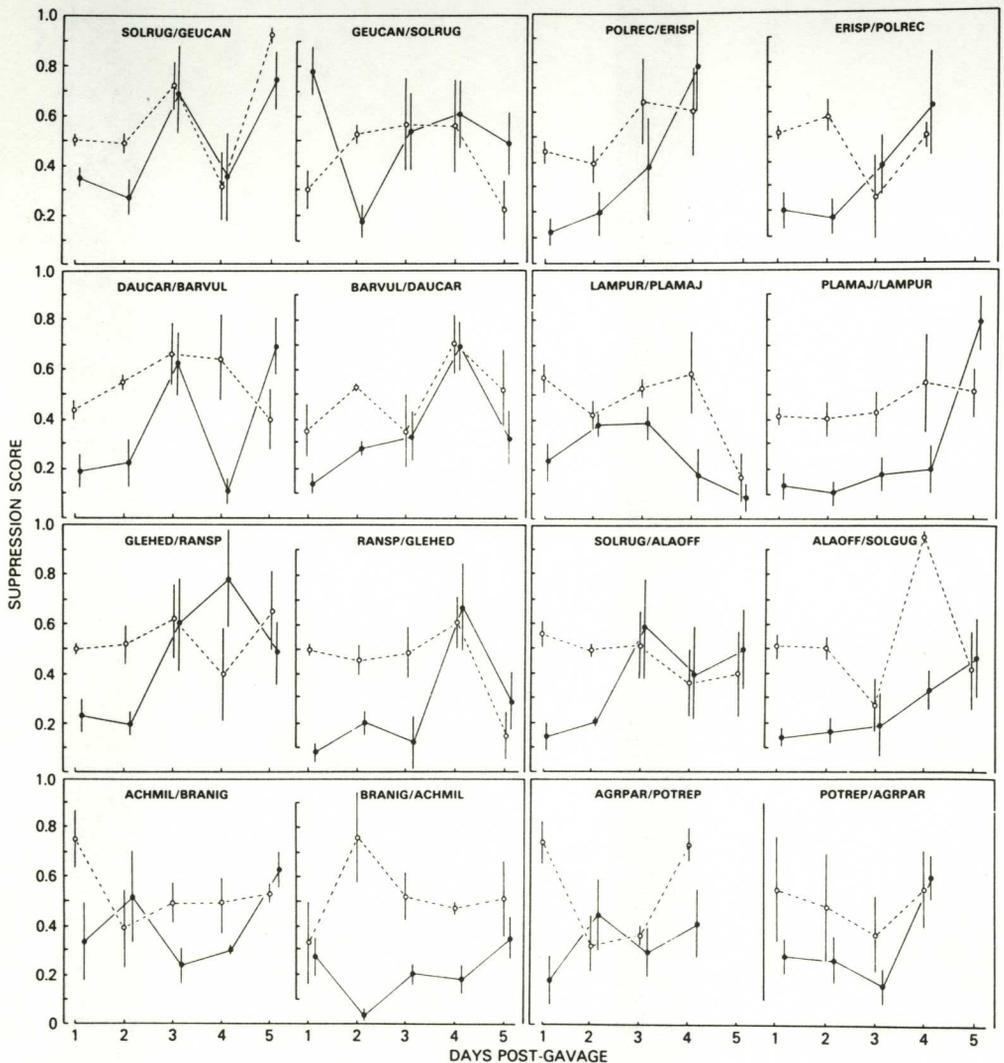


Fig. 5. Results of behavioral two-choice tests. Mean suppression scores were calculated by dividing CS+ by total consumption. Dotted lines represent control groups, while solid lines represent experimental groups. Vertical bars are standard errors of the means.

30 min, of light, and then presented with a cup containing 20 g of food. In the bottom of the food cup was a nylon mesh bag containing fresh leaves of one or another of the plant species. Volatiles from the plant material could diffuse freely into the headspace above the food. After one hour, food cups were removed, and consumption recorded. Plain food was then returned to the cages, and the birds were left undisturbed until the following day. Our aim in presenting fresh plant material during adaptation was to reduce the potential for neophobia during conditioning and test sessions.

Table 4. F values and probabilities for group main effects in eight independent odor discrimination experiments.

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- (1) Agrimonia parafiora vs. Potentilla recta
 $F(1,16) = 23.23, p < 0.001$
 - (2) Achillea millefolium vs. Brassica nigra
 $F(1,16) = 17.05, p < 0.001$
 - (3) Glechoma hederacea vs. Ranunculus
 $F(1,16) = 7.19, p < 0.05$
 - (4) Geum canadense vs. Solidago rugosa
 $F(1,16) = 0.23, p < 0.25$
 - (5) Lamium purpureum vs. Plantago major
 $F(1,16) = 19.98, p < 0.001$
 - (6) Daucus carota vs. Barbarea vulgaris
 $F(1,16) = 10.90, p < 0.01$
 - (7) Erigeron vs. Potentilla reptans
 $F(1,16) = 6.78, p < 0.001$
 - (8) Solidago rugosa vs. Allaria officinales
 $F(1,16) = 30.44, p < 0.001$
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On the day of conditioning, birds were assigned to four groups (5 birds/group), food deprived for 30 min, and then each was presented with a cup containing 20 g of food. In the bottom of the food cup was one or another of two CS+ plant species different from the one presented during adaptation. After one hour, the birds in two experimental groups were gavaged with a toxicant methiocarb/propylene glycol solution (2 mg/kg; Mason and Reidinger, 1983) while the birds in the two control groups were gavaged with propylene glycol (2 mg/kg).

During the first hour of light on each of the five days immediately following the day of conditioning, all groups were food deprived for 30 min and then given 2-choice tests. These tests consisted of presenting two cups, each containing 20 g of food, and one or the other of the plant species presented on the day of conditioning. After one hour, the food cups were removed from the cages and consumption was measured.

Results. The data from each discrimination test were converted to suppression ratios (i.e., CS+ consumption/total consumption), and assessed using three-way analysis of variance (groups, plants, days). The results indicated that the Starlings could discriminate among volatiles from the plants (Fig. 5).

The probability of obtaining seven out of eight statistically significant results by chance was $p < 0.005$. Experimental groups consumed less food when it was associated with the species of plant presented on the day of conditioning. Control groups failed to exhibit such avoidance (Table 4). The one exception was for tests between Geum canadense and Solidago rugosa. This lack of differential consumption might mean that these plants have very similar odors, making discrimination difficult. Alternatively, it might reflect the failure of some experimental birds to learn. Half of

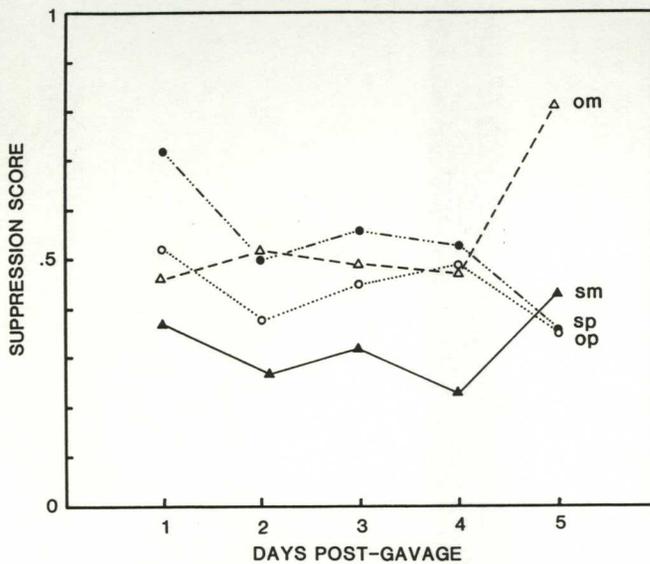


Fig. 6. Results of behavioral tests following bilateral olfactory nerve cuts or sham surgeries. Group om received olfactory nerve cuts and methiocarb intubations; Group sm, sham surgeries and methiocarb intubations; Group sp, sham surgeries and propylene glycol intubations; and Group op, olfactory nerve cuts and propylene glycol intubations. Suppression scores were calculated as previously described.

the experimental birds showed non-differential consumption, the other showed clear preferences for CS- over CS+ on all test sessions.

While birds could be trained to respond and discriminate among plants, we still had not demonstrated that such behavior was mediated by a nasal chemosensory cue. To answer this question, another series of behavioral experiments was undertaken with birds who had been given either bilateral olfactory nerve cuts or sham surgeries.

Starlings were assigned to two groups ($n = 10/\text{group}$). The birds in one group were lightly anesthetized with Equithesin, and placed in a headholder. The olfactory nerves under the bony orbital walls were exposed. The nerves were lifted slightly and a small section (approximately 1 mm) was removed. The cut ends of the nerves were folded back, the cavity packed with gelfoam, and the skin closed with cyanoacrylate glue. The birds in the other (sham) group were treated similarly, except that the nerves were not disturbed under the orbital walls. All of the birds recovered from surgery within 1 h of Equithesin injection. Beginning two days after surgery, the birds were given four days of adaptation to food deprivation. Five of the birds given olfactory nerve cuts, and five of those given sham surgeries were given a pairing of a plant species and methiocarb intubation. The other birds were given a pairing of the plant and propylene glycol intubation. On each of the four days following the day of conditioning all of the birds were given two-choice tests, as previously described.

Analysis of the results indicated that while sham operated methiocarb-gavaged birds exhibited avoidance of volatiles from the plant associated with sickness, methiocarb or propylene glycol gavaged olfactory nerve cut

birds and propylene glycol gavaged sham birds failed to exhibit differential behavior (Fig. 6). We took these results as evidence, at least for the species of plants tested, that avoidance was mediated primarily by olfactory cues.

CONCLUSION

North American passerine species that reuse nest sites are more likely to incorporate fresh green vegetation into their nests than species who do not reuse nests. Several explanations for the use of green vegetation have been offered, including sexual selection (Collias and Collias, 1974), crypticity (Skutch, 1976), and the possibility that green plants possess structural or thermal characteristics which enhance nestlings' chances for survival (Kern, 1984). In the present paper, we have suggested an additional possibility; that the chemical defenses of green vegetation are being used by birds to control ecto-parasites and bacterial pathogens. Several lines of evidence support this possibility. For example, Sengupta (1981) reported that house sparrows (*Passer domesticus*) incorporate margosa (*Azadirachta indica*) leaves into their nests and that these leaves are used in preference to other available vegetation. Isolated fractions from leaves not only are repellent to arthropods, but also inhibit oviposition on treated surfaces. The suspected active chemicals are a steroid, β -sitosterol, and a phenolic, quercetion (Ambasta, 1980). Similarly, nuthatches smear nest cavities with pine resin (Bent, 1965), a material containing a variety of monoterpenes (e.g., alpha and beta pinene) known for their antiseptic and toxicologic properties (Rosenthal and Janzen, 1979). Finally, we have demonstrated that Starlings choose particular species of plants for inclusion in their nests, and that preferred plants exert fumigant effects in laboratory experiments. While the plant compounds exerting these effects remain unknown, the available evidence suggests that they may be mono- and sesquiterpenes, including known juvenile hormone analogs for ecto-parasites. We speculate that these analogs may act to control ecto-parasite populations by delaying maturation, and hence reproduction. The implied importance of plant derived juvenile hormone analogs can be appreciated when one considers that the per capita blood loss for a brood of four in a heavily infested nest can be 3.5 percent of the daily blood production of the chicks (Powlesland, 1978).

The underlying bases (e.g., unlearned preference, breeding experience, imprinting, observational learning) of plant selection and use remain unclear, although proximate mechanisms of discrimination likely include visual and chemical cues. We present electrophysiological and behavioral evidence that volatile cues could be used to detect and discriminate among plant species. In the field, we speculate that birds may visually orient towards patches of vegetation. However, once in a patch, vision alone probably would not serve for accurate discrimination, since the use of plant morphology as the sole criterion for plant selection would not guarantee a bird access to a particular chemical profile. Specifically, there is high variability of chemical profiles found within plants, even of the same species (Parks, 1974). An alternative is that, once in the patch, particular species of plants (and perhaps even particular individual plants) may be selected on the basis of a volatile cue. When foraging for green vegetation, Starlings pluck bits of plants, turn them in their bills, and then either accept or reject them (Clark, pers. obs.). These manipulations may facilitate retronasal access of plant volatiles to the olfactory epithelium (and possibly the detection of any salient taste cues that might be present). Welty (1975) has suggested that the large size of the internal nares in avians may serve to enhance access to the nasal cavity. From an ecological perspective, we suggest that chemicals represent a logical source of cues for plant selection. It would appear both logical and efficient to choose

plant materials on the basis of cues (i.e., chemical volatiles) that may be directly responsible for insecticidal and anti-pathenogenic effects.

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