Table 2. Effects of type and amount of soil on the synergism of parathion toxicity to mosquito larvae (Aedes aegypti L.) by atrazine in 20-ml water solutions. After the soils were added to the pesticide solutions the samples were shaken vigorously before the larvae were added. Results are means of three replicated tests.

Pesticide and concentration (ppm)	24-Hour mortality (%) of larvae in water with				
	No soil	Sand		Loam	
		1 g	5 g	1 g	5 g
Atrazine (10)	0	0	0	0	0
Parathion (0.015)	24 ± 7	16 ± 7	2 ± 2	7 ± 0	0
Atrazine (10) + parathion (0.015)	62 ± 8	42 ± 10	2 ± 2	22 ± 4	0
Atrazine (20)	0		0		0
Parathion (0.30)	93 ± 6		62 ± 8		0
Atrazine (20) + parathion (0.30)	98 ± 4		76 ± 4		38 ± 10

residues declined rapidly; while the toxicity was higher in the presence of atrazine, the curve obtained with atrazine present drops off parallel to the curve for parathion alone. The concentration of the insecticide in the soil after 4 days was 0.24 ppm, which is the lower margin of the killing range of parathion in the loam soil.

Although the toxicity of parathion reached a low point on the second day after it was added to the soil (Fig. 2B), gas chromatographic analyses of the soils indicated that the parathion concentration did not change during the first 3 days (0.35 ± 0.015 ppm), and it was only on the fourth day that the low concentration of 0.24 ppm was determined. This suggests that by the second day enough insecticide had been bound to the soil particles to reduce its toxicity and prevent it from being as effectively synergized as freshly deposited residues. Extraction of these soils and exposure of fruit flies to dry residues of the extracts confirmed these results. Extracts of the soil samples for the first 3 days caused high insect mortalities (83 to 98 percent after exposure for 20 hours to dry residues representing 1.14 g of soil), while the extract from soil incubated for 4 days caused considerably lower insect mortality (46 percent).

The synergistic effects of atrazine with parathion in water or in water with soil were measured with mosquito larvae (Aedes aegypti L.). In all experiments, 15 third-instar larvae were placed in 20 ml of tap water, previously treated with parathion, atrazine, or both in water solutions. To study the effects of soils in water, 1-g or 5-g portions of the sand or the silt loam were poured into the pesticide-treated water in vials; the vials were centrifuged at 100g for 5 minutes, and then mosquito larvae were introduced. To study the effects of water turbulency, tubes containing water and soil were shaken and vigorously swirled for 1 minute, then centrifuged at 100g for 5 minutes, and mosquito larvae were introduced. Mortality counts were made after 24 hours.

Table 1 summarizes the results obtained after exposure of the larvae to water (with and without soils) treated with atrazine (10 ppm), parathion (0.015 ppm), or both. Under these conditions soil reduced the effectiveness of the insecticide. Atrazine, which is not toxic by itself, significantly increased the toxicity of the insecticide. Shaking and mixing the soil and water significantly reduced the toxicity of the insecticide with both sand and loam, apparently by increasing the binding of the insecticide by soil particles. The synergistic effect of atrazine in water was not affected by soil under nonturbulent conditions, but was significantly reduced under turbulent conditions. In nature, soil is mixed with water in the runoff into lakes and streams, whereas bottom mud in deepwater lakes is not likely to be mixed with the upper water layers, even after surface turbulency. Therefore, both conditions simulated in these experiments could prevail in nature and affect the biological activity of pesticides.

Table 2 shows the results obtained with different amounts of soil in the water. In these experiments, 1 or 5 g of the sand or the silt loam was added to 20 ml of pesticide-treated water and the mixture was shaken. With the larger amount of soil, the biological activity of parathion as an insecticide and of atrazine as a synergist was significantly reduced. In solutions with no soil and the relatively high parathion concentration of 0.3 ppm, 93 percent of the mosquito larvae died within 24 hours, yet these solutions were rendered nontoxic by being shaken with 5 g of the loam soil. With atrazine added to this system, however, 38 percent of the mosquito larvae died. Insecticides, therefore, can be more or less toxic, depending on their concentrations, the presence of insecticide synergists, and the environmental conditions.

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Target Structure and Echo Spectral Discrimination by

Echolocating Bats

Abstract. Echolocating bats can use sonar to discriminate among targets which reflect echoes differing in spectral distribution of energy but not in overall intensity. They can detect differences smaller than 1 millimeter in fine target structure. Bats may be capable of classifying targets from echo spectral signatures and might thus be able to distinguish among flying insect prey by sonar.

Microchiropteran bats use a biological sonar system, echolocation, as a partial substitute for vision in their nocturnal lives (1). With echolocation bats can detect, locate, and track flying insects (1, 2) and discriminate among mealworms and plastic spheres or disks propelled into the air (1, 3). Echoes from such airborne targets differ in spectral composition (3, 4), and there is evidence that bats may be able to perceive target features from echo spectral cues (5). This report describes an experimental study of discrimination of target echo spectral differences by echolocating bats. The results show that bats can indeed discriminate among targets on the basis of echo spectral information.

Two insectivorous bats of the species *Eptesicus fuscus* were trained in the experiments described here. The bats were blinded under ether anesthesia in order to prevent the possible use of visual cues in discriminations and deprived of food until their weights declined to 14 to 16 g from more than 20 g at capture.

Each target consisted of a square Plexiglas plate (7.3 by 7.3 cm) with a thickness of 2.2 cm. The targets were simultaneously presented in pairs separated by 40° and mounted 30 cm away from the bat's starting position. The bat was rewarded with food for moving toward the correct target (6). The spectrum of echoes from each target was manipulated by drilling 24 holes, in a 5 by 5 array with the center hole missing, partway through the face of the Plexiglas square. On any given target the holes were of the same depth, and they had a diameter of 10.5 mm. The positive stimulus or correct target was always a square with holes 8.0 mm deep. (The hole depth describes the distance from the front surface to the point or apex of the hole's 30° conical bottom.) Five different targets were sequentially paired with the target containing 8.0-mm holes; the depths of the holes in these negative stimuli were 6.5, 7.0, 7.2, 7.6, and 8.0 mm. Two 8.0-mm targets were used interchangeably to guard against some minor feature of any individual target serving as a cue to the discriminations.

After being trained to discriminate the initial target pair with 8.0- and 6.5-mm holes, each bat worked through this sequence of stimulus pairs for determining the smallest discriminable difference in the depth of the holes. Fifty trials were conducted for each pair before proceeding to the next pair in the series. Warm-up trials were conducted at the beginning of each day's session. Forty to fifty trials were carried out each day.

The bat was first trained to respond to the 8.0-mm target located close to it (15 to 20 cm away), and not to respond to a flat target (with no holes) located farther away (30 cm). Gradually the 8.0-mm target was moved farther back until the bat could discriminate between the 8.0-mm and the flat targets when both were 30 cm away.

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Fig. 1. (a) Discrimination performance of two *Eptesicus* as a function of the difference between the depths of the holes in the sonar targets. (b) Digitally plotted waveform of a representative *Eptesicus* sonar sound recorded



during discrimination of 8.0- and 7.2-mm targets. Notice the distortions in the last three-quarters of the signal caused by strong harmonics. (c) Comparison of the amplitude spectrum of the signal in (b) (left ordinate) with the behavioral audiogram of *Eptesicus* (right ordinate) (10). Spectral amplitude is relative to the amplitude at 30 khz; sound pressure is relative to 1 dyne/cm².

On succeeding days thereafter, several 6.5-mm holes were added to the flat target until the bat was finally discriminating between the 8.0- and 6.5-mm targets. Training to discriminate between the 6.5- and 8.0-mm targets took about 6 weeks with 10 to 30 trials per day.

The results of the hole-depth discriminations are shown in Fig. 1a. As the graph indicates, both bats could readily detect the difference between 8.0-mm holes and either 6.5- or 7.0-mm holes. As the difference in hole depth declined below 1.0 mm, the bats' performance deteriorated until they performed at chance levels when presented with a pair of stimuli both containing 8.0-mm holes. If we use the arbitrary criterion of 75 percent correct responses to summarize the data, Eptesicus can detect differences as small as 0.6 to 0.9 mm in the depths of the holes.

The orientation sounds used by the bats for the hole-depth discriminations were recorded with a Brüel and Kjaer model 4135 condenser microphone and a Pemtek model 110 magnetic-tape recorder. They were examined with a General Radio high-speed transient analyzer (7) and analyzed with the Fast Fourier Transform algorithm in a PDP-11/40 computer. For digital processing, the bat signals were sampled at a rate of 512 khz (10-bit accuracy) and stored in a block of 1024 memory words.

The waveform of a representative bat sonar signal from the hole-depth discrimination experiment is shown in Fig. 1b. The sounds are generally similar to the orientation sounds used by Eptesicus in size, shape, or distance discrimination experiments conducted with the same two-choice procedure (6, 8). The signals are frequencymodulated (FM) downward with durations of 1 to 2 msec. The first harmonic sweeps from about 50 to 25 khz, and strong second and third harmonics are present (see Fig. 1b). The amplitude spectrum of the illustrated signal is shown in Fig. 1c. The bandwidth of the sounds typically is about 70 khz, which is higher than the bandwidth of sonar sounds used by Eptesicus for target range discrimination (8) because of the presence of stronger harmonics. Note the sharp spectral variations around 50 khz and also between 75 and 100 khz. These originate in the overlap of harmonics such that a given frequency, for example 50 khz, occurs twice in the bat's sound, once in the first harmonic and again in the second harmonic (see Fig. 1, b and c). These variations in spectral amplitude occur with a frequency spacing equal to the reciprocal of the time separation between repetitions of a given frequency in successive, overlapping harmonics

and can be examined by "cepstrum" analysis of the spectrum (9). Such cepstrum oscillations in the spectrum of the bat's sound are often found for *Eptesicus*, and they are especially prominent in the sounds used for discrimination of hole depths.

Figure 1c shows the behavioral audiogram of Eptesicus (10) for comparison with the spectrum of one of the bat's orientation sounds. The actual echoes of bat sounds reflected back to the bat from each of the targets have peak sound pressures of about 0.1 dyne/cm². The bat could certainly have heard energy in echoes at frequencies as high as 90 khz.

The acoustic properties of each target were measured by generating FM sounds (sweeping from 100 to 10 khz in 1.5 msec) from an electrostatic loudspeaker (11) aimed at the center of the target and located in the bat's observing position. Echoes of these test sounds reflected back from the target were picked up with the Brüel and Kjaer condenser microphone and analyzed digitally. Test echo spectra were ensemble-averaged (20 samples) to reduce interference from electronic noise in the microphone's preamplifier, and the results were made available as an echo amplitude spectrum for each of the targets used in the experiment. The spectrum for each target with holes was subtracted from the spectrum for the flat target to obtain acoustic absorption spectra showing the effects of the holes alone, not confounded with the acoustic properties of the loudspeaker, microphone, or air transmission pathway. The absorption spectra are given in decibels with respect to the spectrum of the flat target in Fig. 2, a procedure commonly used for such data (3-6). Test echoes were required for measuring the acoustic properties of the targets because echoes of the bat's sounds were too close to the noise level of the microphone system for very reliable analysis.

The presence of the holes in the targets introduces several absorption peaks in the echo spectrum for each target (see Fig. 2). In all the targets a strong spectral "notch" is present between 30 and 40 khz, another between 45 and 60 khz, and several more at higher frequencies. The notches between 30 and 60 khz seem particularly closely related to the depths of the holes in the targets, with deeper holes vielding somewhat lower-frequency notches. Spectral notches above 70 khz



Fig. 2. Acoustic absorption spectra for the targets used in the hole-depth discrimination experiment. The echo amplitudes are relative to those from a smooth target.

are somewhat variable in position and not so simply related to the depths of the holes. The bat's discrimination performance improves as the difference in hole depth becomes larger, which suggests that if the bat uses spectral cues to perform the discriminations, information between 30 and 60 khz must be of greatest importance.

Overall echo intensity differences among the targets were obtained by integrating the areas under the envelopes of test echoes from each target or by integrating the test echo spectra. Differences in overall echo intensity between members of pairs of targets presented to the bats were between 0.1 and 0.5 db with respect to the intensity of the echoes from the 8.0-mm target (Fig. 2A). It seems most unlikely that overall echo intensity could have been a cue for the bats' discriminations.

Echolocating bats determine distances to targets in terms of the travel times of outgoing and returning echoes, processing echoes in a neural mechanism that performs as accurately as an ideal sonar receiver (8). Apparently the bat can extract from the echoes not only time-domain (range) information, but also frequency-domain or spectral (shape) information, judging from the hole-depth discriminations reported here. The same signal-processing operation may underlie both temporal and spectral resolution of echoes within the bat's auditory system, just as timedomain and frequency-domain displays of waveforms are mutually related through the Fourier transform. The echolocation signals of bats apparently represent a class of waveforms that are optimal for characterizing linear systems such as sonar targets (12). It may be of considerable interest that the bat is capable of quickly and efficiently evaluating target transfer functions by an ideal mechanism (8, 9, 12, 13).

The data reported here indicate that Eptesicus can make judgments based on echo spectra. The bat may be able to learn relations between particular naturally occurring echo sources (flying insects, for example) and the spectral signatures of their echoes. If so, the bat could perceive the shape of a target in terms of the "quality" of echo spectra and might be capable of identifying objects or distinguishing among flying insect prey on the basis of echo spectral cues, although they may be able to use other cues for the same task.

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