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FIELD TRIALS OF THE WOLF TRAP

ENGINEERING BREADBOARD

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INTRODUCTION

In September 1964, Ball Brothers Research Corporation of Boulder Colorado delivered to the University of Rochester an advanced breadboard of the Wolf Trap which incorporated a number of the salient features necessary for space flight and automatic operation. This completed the first phase of their subcontract with the University of Rochester to instrument the concepts of the Wolf Trap for eventual inclusion in a Martian mission. Among the constraints met in this model were sterilizability, sensitivity of detection, automatic sample collection and automatic readout, and packaging to allow an eventual field trial of the instrument.[1] The main constraints not met in this model were shock and vibration resistance, vacuum tolerance, and complete miniaturization and weight reduction. These are being attended to in the second phase flight engineering model.

Upon delivery to the laboratory, tests were undertaken to familiarize ourselves with the operational characteristics of the Wolf Trap and to select a medium which might be used as a culture medium in terrestrial experiments. Although further information must still be obtained in many areas it was felt that there was no advantage to be gained in postponing a field trial from the Spring of 1965 until the next Fall when the weather would again be comfortable for working. We wished to learn two things from the field trip. First, we wanted to verify the automatic operation of the Wolf Trap under conditions where the normal backup of laboratory equipment was not available. Secondly, we wished to subject the instrument to an environment which would challenge its ability to detect microorganisms in very low concentrations. Two sites were selected for this test, a desert site and a mountain site. The field tests were undertaken with the support of personnel from the Jet Propulsion Laboratory,[2] and Ball Brothers Research Corporation.

SITE SELECTION

Two types of conditions were selected as being desirable for testing the Wolf Trap: cold-dry and hot-dry. For the cold-dry environment our choice was the high altitudes of the White Mountain range in eastern California. In part we were guided by the availability of the White Mountains Research Station which could serve as a convenient base camp. However, the presence of snow on the range at the time of testing precluded operations at the high altitude. Consequently, this report will be devoted exclusively to the results of the hot-dry testing on the Mojave desert in the vicinity of Death Valley National Monument, California.

The place selected for the tests was on the Dumont sand dunes[3] (Figure 1) immediately adjacent to an extensive plain almost devoid of vegetation (Figure 2). It was felt (and later confirmed) that under these conditions there would be an extremely low microbial flora. In the first place the organic material to support microbial growth would not be available in the virtual absence of higher plants. Secondly, any water which might support growth and metabolism of microorganisms rapidly evaporates at the high temperatures and low relative

humidity of this area. Notwithstanding these conditions, a surprising number of higher organisms were observed. Two types of higher vegetation were noted. On the plain leading up to the sand dunes were small plants which spread no more than eight or ten centimeters and which grew only a few centimeters above ground level. These were found five to ten meters apart where they were most concentrated, although in many areas one could walk many meters and not find plants even when they were being sought. The other conspicuous plant form was low shrubs, the closest one to the test site being about 200 meters away. The next closest was at least as far away in the opposite direction, while 400-500 meters away was a group of perhaps two dozen shrubs ranging in size up to 1½ meters tall. On the plain it was not difficult to discover ant colonies located ten to fifty meters apart. At dusk a black beetle emerged from the sand and foraged over the dunes during the night, burrowing back into the sand the following morning. The only other insect observed in any abundance was a small dun colored cricket which could not be seen at night, perhaps only because of its lack of contrasting color, but which was seen each morning burrowing into the sand in large numbers. The beetles seemed to subsist upon the bits and pieces of chaff that must have blown from the distant bushes but there was no indication of what the ants or the crickets subsisted upon. The only vertebrates observed were two lizards. No attempt was made to identify the plants or animals. The region clearly was not devoid of life in spite of its exceptionally hostile aspect.

TEST PROCEDURES

A. Medium:

The medium selected for the tests contained in one liter:

$MgSO_4 \cdot 7H_2O$, 0.015 g	KNO_3, 0.0375 g
Na_2MoO_4, 0.00075 g	Soil Extract, 25 ml
$MnCl_2 \cdot 4H_2O$, 0.00075 g	Yeast Extract, 0.1 g
$CaCl_2$, 0.0015 g	Distilled H_2O, 975 ml

The medium reservoir for channel 1 was filled and the entire assembly autoclaved before departing from the Jet Propulsion Laboratory. Channel 2 cannot be inoculated or filled automatically so the medium was introduced aseptically into the pre-sterilized chamber from a sterile bottle just prior to manual inoculation at the test site. The experiment was then placed on the sand in an area that had been undisturbed by our movements.

B. Sample induction:

With all of the functions on the operating console in the automatic mode and the pickup time set for one second, the automatic sequencing switch was tripped. Just prior to taking the field trip, Ball Brothers had modified the pickup to enhance its efficiency greatly. We therefore felt that one second would be an adequate pickup time. However, in the trial runs while flushing the pickup we had noticed that a considerable advantage was

gained when the pickup nozzle was moved in the sand. Therefore, to boost the pickup efficiency and assure an adequate sample during the one-second pickup, the entire experiment was slowly rotated with pickup nozzle submerged just under the surface of the sand. The sample was later estimated to be approximately 10 mg. Channel 2 was inoculated by hand with a sterile scoop after the sequencing had begun.

C. Medium dump:

The disposition of the medium into the chamber did not function properly and it was necessary to depress it manually in order to get the medium from its reservoir into the chamber. This was not entirely unexpected. The two departures from automatic operation - moving the pickup and manually activating the medium dump - are discussed below.

D. Data recording:

The data were recorded by hand at 20 to 30-minute intervals throughout the test period rather than automatically as has been the practice in the laboratory. This choice was dictated primarily by a desire to reduce to a minimum the amount of equipment that had to be carried in the field.

RESULTS

Figure 3 shows the results from the two channels. Channel 2 which was automatically inoculated began a slow increase from the fifth hour to the eighteenth hour. With automatic recording the sensitivity would have clearly shown whether this represented random fluctuations or clear-cut growth. Whatever the case, at 18 hours there was a clear onset of growth which continued exponentially to approximately the 30th hour followed by continued growth at a gradually decreasing growth rate. From the fifth to the twelfth hour channel 1 also increased and at a slightly greater rate than channel 2. This increase appears to be growth. Thereafter there was a rapid settling out in this channel which was only offset at the 18th hour when an increase began which seems to have parallelled that in channel 1. An unexplained drop at 25 hours was followed by a further increase which seems to continue parallelling the channel 1 response.

A sample of soil taken from an undisturbed area about 20 feet from the test site was plated in the laboratory to estimate the numbers of viable organisms. The medium used for the plate counts was the same as that used in the growth chamber. Figure 4 shows a breakdown of the microbial count. The total estimate of viable organisms is approximately 8×10^4/gm. This should be compared to meadow or garden soils which typically have 10^8 - 10^{12} organisms per gram. Of the desert organisms consistent with earlier findings on desert soils,[4] almost 50% were filamentous actinomycetes. Based on this count approximately 400 bacteria were inoculated in the two chambers.

DISCUSSION AND CONCLUSIONS

Although the hand inoculated channel 2 gave rather puzzling and ambiguous results, the automatic channel 1 showed clearcut exponential growth after the 18th hour, starting from a low inoculum. Of the 400 or so organisms which

may have been inoculated certainly not all of them contributed to this growth. It is a common observation that there is among microorganisms, as well as in higher ecological systems, a succession of organisms in time with one organism predominating for a time followed by another and yet another one until some equilibrium is reached. It is impossible to tell with the information at hand which organism may have contributed to the growth observed under our conditions. If we generously estimate that one half of the inoculated bacteria were of the type which contribute to the growth that was seen after the 18th hour, and if we assign a generation time of two hours based on the slope of growth curve, we might have expected to see growth at 6 hours. The discrepancy between this figure and the observed time presumably results from a lag period before growth is initiated.

We have drawn several important conclusions from this trip. On the positive side, it is possible with the Wolf Trap in reasonable lengths of time to demonstrate the presence of microorganisms from inhospitable sites by observing their growth. It would be desirable to duplicate these tests in a cold-dry, high altitude environment and this will certainly be undertaken. An adequate interpreatation of some of the results must depend upon a more thorough understanding of the types of organisms which are present in the soil which is being tested, as well as a clear understanding of the principles which govern microbial ecological systems. Continued study of the relation of microorganisms to their soil habitat are under way in our laboratories, but we feel that added emphasis is desirable. In support of this, soil samples were collected from Death Valley National Monument following the field trials.

The greatest weakness which was shown by the experiment lies in the area of mechanical design. The pickup, although it provided sufficient suction to collect a large soil sample, did not collect this sample unaided. Next to dust, loose sand is as nearly ideal a material for pickup as can be found. In a more difficult environment, as, for example, randomly distributed rocks with only occasional patches of soil between the rocks, this pickup design would be inadequate. Our observations in the field support the belief that we have held for a considerable time now, that the success of this (or any) experiment will rest ultimately on the adequacy of the pickup system that is used. In the redesign of the Wolf Trap into a flight engineering model special attention is being given to the pickup so that it will be more versatile. However its success may rest upon eventual incorporation of a roving pickup probe which will seek out favorable places to sample.

We had already experienced unsatisfactory operation of the medium dump during laboratory tests. Unfortunately, it completely failed to operate in the field. We were naturally disappointed that completely automatic operation was not successful, but the failure of the medium dump will not influence future design since another approach had already been developed for the flight engineering model.

1. Engineering Breadboard Model, Wolf Trap Microbe Detection Device. Final Report F65-6. Ball Brothers Research Corp., Boulder Colorado.

2. Charles R. Weston, University of Rochester; Gerald A. Soffen & Robert Rolofson, Jet Propulsion Laboratory; Louis Ried & Dale Buckendahl, Ball Brothers Research Corp.

3. The Dumont Sand Dunes are located near Death Valley National Monument route 127, 35 miles north of Baker, California. This site is about 5 miles from the Ripple Dunes adjacent to route 127 where Gulliver field tests were conducted (G.V. Levin & A.H. Hein 1964. Gulliver and Diogenes - Exobiological antitheses. Presented at the Fifth International Space Science Symposium COSPAR, Florence Italy, May 1964).

4. Mishustin 1956. Soils and Fertilizers 19:385(Cited in Burgess, Alan 1958. Microorganisms in the Soil. Hutchinson and Co., London.).

Figure 1. Wolf Trap (arrow) on sand dunes during testing.

Figure 2. Looking from Wolf Trap test site across the desert plain.

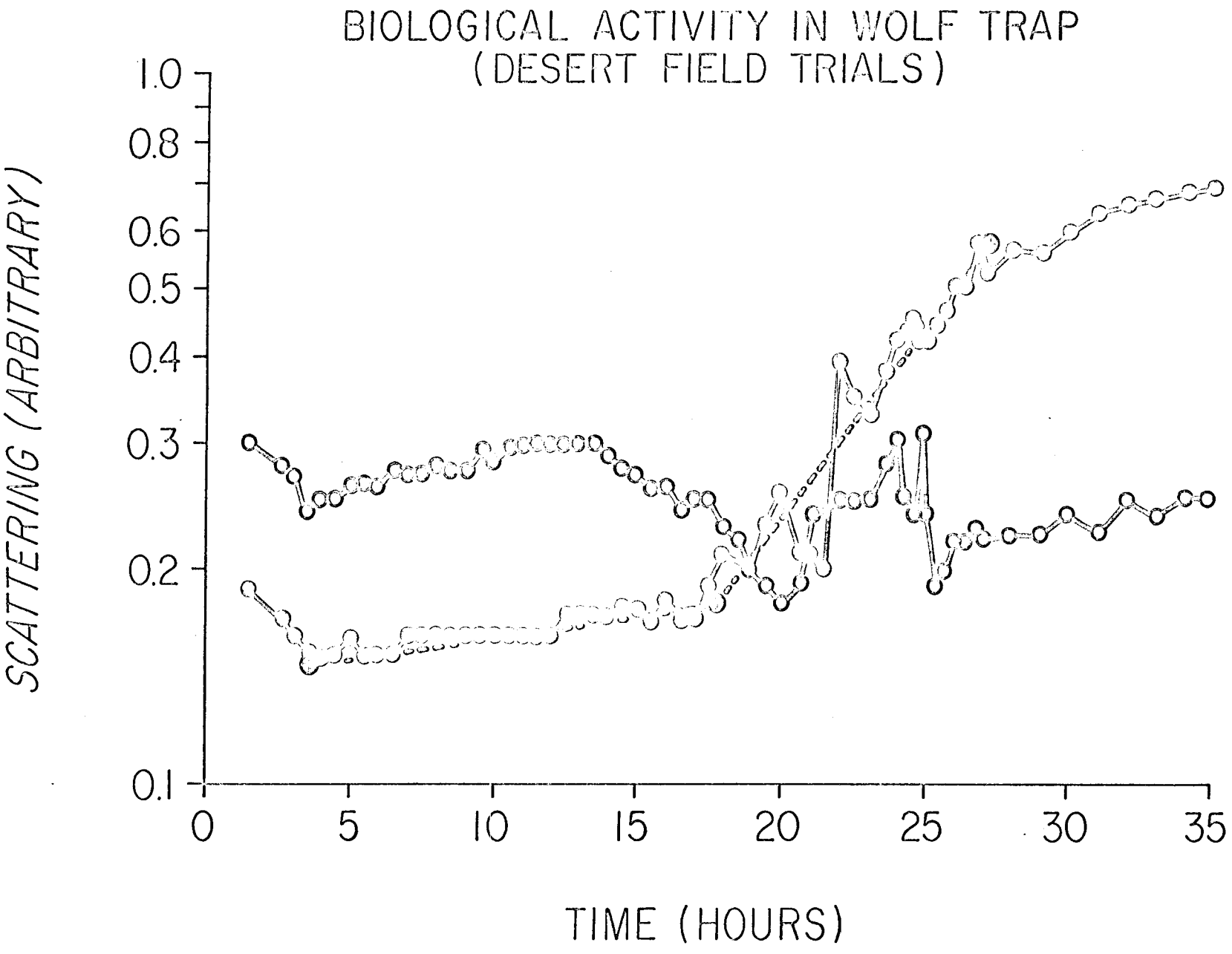
BIOLOGICAL ACTIVITY IN WOLF TRAP
(DESERT FIELD TRIALS)
SCATTERING (ARBITRARY)
1.0
0.8
0.6
0.5
0.4
0.3
0.2
0.1
0
5
10
15
20
25
30
35
TIME (HOURS)

Figure 4. Microbial plate counts of desert sand dunes from test site of desert field trial of the Wolf Trap.

Results of 10^{-3} Dilution. (Parentheses enclose actinomycete counts when they were determined.)

	Replicate Weighings			
	A	B	C	D
	74 (38)	81 (45)	101 (46)	59
	64	74	106	42
	90	73	107	92
Average	76	76	105	64

Grand average..... 80.25 ± 5.22
Estimate of microbial numbers.. 8.0×10^4

Analysis of Variance

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	Level of Significance
Weighings	2,657	3	886	5.53	0.025>0.05
Platings	736	2	368	2.30	0.05>0.25
Residual	960	6	160	-	-
Total	4,353	11			