opening in red light, inhibit the production of ethylene (Table 1).

Gibberellic acid, which consistently promoted the opening in red light (*1*), did not inhibit ethylene production, and its effect must therefore be regarded as independent of ethylene (Table 1).

Red light also induced a consistent increase in the rate of production of CO_2, as shown by data given in Table 1. In view of the previously mentioned evidence that respiratory CO_2 partly antagonizes the inhibitory action of endogenously produced ethylene in light-treated hooks, it seems probable that the promotion of CO_2 production by light may also be involved in the induction of opening by light. The effect of light on output of CO_2 is not simply a consequence of promotion by light of the growth of cells in the elbow, because the effect of light on respiratory CO_2 output was just as strong in a concentration of auxin that completely inhibited opening of the hook (Table 1).

It seems clear that ethylene, and possibly CO_2 also, serve as natural growth regulators in the hook-opening response of the bean hypocotyl. Regulation by ethylene may be of particular value in terms of the biological role of the hook-opening response. As long as the hook is growing underground the buildup of its endogenous ethylene that should result from confinement in soil would tend to prevent opening. Thus, opening could occur only after emergence from the soil, even though morphogenically effective intensities of light might penetrate some distance below the surface of the soil.

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6. In experiments of this type, about 20 hooks were supported by their bases in agar in a closed vessel (gas volume, about 250 ml.) Auxin treatment was given by applying, at the apical cut end of the hook, an agar block containing IAA.
7. S. P. Burg and E. A. Burg, *Plant Physiol.* **37**, 179 (1962).
8. In Table 1, gas output was measured by incubating hypocotyl tissue segments for 19 hours in 5 ml of test medium in a sealed 60-ml flask, a sample of air being withdrawn for gas chromatography at the end of the period. Red light treatment was continuous illumination, during incubation period, with about 250 erg cm^{-2} sec^{-1}, wavelength >600 mμ. Angle of opening of red light controls was 95° to 110° in different experiments from which data on hook opening quoted in Table 1 were drawn.
9. Similar results with pea epicotyls have been obtained by J. D. Goeschl (private communication).

10 February 1967

Dieldrin: Degradation by Soil Microorganisms

Abstract. *An attempt was made to discover microorganisms that degrade dieldrin, an extremely stable chlorinated hydrocarbon insecticide. Examination of more than 500 isolates from soil that had been heavily contaminated with various insecticides revealed the existence of a few microbes that are very active in degrading this compound to various metabolites.*

Certain chlorinated hydrocarbon insecticides are known to be very stable and to persist in soil for many years (*1*). Among them, dieldrin is the most stable and hazardous insecticide in our environment (*2*); this persistence suggests that it is of low biochemical reactivity and also makes plausible the view that its potent effect upon insects and mammals depends upon a physical complex with their nervous systems, rather than a chemical reaction (*3*). Most chlorinated hydrocarbon insecticides have little effect upon bacterial and fungal growth (*4*), and many microbial changes brought about by application of these insecticides to soil may be attributable to other secondary effects (*5*).

Recently, Chacko *et al.* (*6*) demonstrated that several actinomycetes can degrade 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), but no microorganisms tested by these authors were capable of degrading dieldrin. Korte *et al.* (*7*) also found a number of microorganisms that slowly degraded all representative chlorinated cyclodiene insecticides except dieldrin. Studies on the degradation activity of dieldrin by biological systems other than microbes have been equally unsuccessful, although there are reports that dieldrin can be degraded very slowly in vivo by some mammals (*8*) and insects (*9*). None of these degradation activities can be regarded as significant enough to reduce the actual toxicity of dieldrin by these systems, for the amount of metabolites in each instance was extremely small.

In this study we attempted to survey the degradation activities of various microbial isolates from samples of insecticide-treated soil in the hope of finding certain microorganisms that degrade dieldrin.

Soil samples that contained dieldrin-degrading microbes were obtained from five major locations: two from apple orchards in northeastern Ohio (Tope's orchard, Fredericksburg, and Synder's orchard, Wooster); two from the dieldrin factory yards of Shell Chemical Company near Denver, Colorado; one from an orchard area near the University of Wisconsin, Madison; and two from a peach orchard near Fort Valley, Georgia.

Microorganisms were isolated from soil samples by adding 1 g of soil to 99 ml of sterile water and shaking the mixture vigorously for 3 minutes. From this stock, serial dilutions of 10^{-2} and 10^{-3} were made in sterile petri dishes and mixed with soil-extract agar of the type described by Allen (*10*). Plates were incubated at 30°C for 4 days; they were then examined, and individual colonies were selected and streaked singly on soil-extract agar. After incubation, an additional and similar transfer was made to ensure purity of the culture. For dieldrin incorporation, each isolate was first inoculated in 10 ml of a solution of yeast extract and mannitol, as described by Fred and Waksman (*11*), and maintained at 30°C for 57 hours. This mixture was then incubated with 0.01 μmole of C^{14}-dieldrin (universally labeled, specific activity 9.4 mc/mmole), added with 10 μl of acetone, in a screw-capped 20-ml test tube at 30°C for 30 days without shaking. The reaction was stopped by adding a 0.1-ml portion of 20 percent trichloroacetic acid to the tube, and the contents were immediately extracted twice, each time with an equal amount of chloroform that had been dried over anhydrous sodium sulfate. The distribution of radioactivity between the aqueous and solvent phases was re-

Table 1. Soil microorganisms with high dieldrin-degrading activities. Data are expressed in percentages of applied dieldrin.

Culture No.	Soil source	Microorganism	Metabolites produced from			
			Microorganisms + dieldrin		Original soils + dieldrin	
			Water-soluble	Solvent-soluble	Water-soluble	Solvent-soluble
12	Snyder	*Trichoderma viride*	1.9	40.8	1.4	6.0
27	Tope	*Pseudomonas* sp.	52.5	18.6	3.0	8.2
33	Shell	*Pseudomonas* sp.	33.4	12.6		
34	Shell	*Pseudomonas* sp.	14.1	31.2		
41	Shell	*Trichoderma viride*	1.7	14.8		
94	Snyder	*Pseudomonas* sp.	12.0	10.8	1.4	6.0
103	Shell	*Pseudomonas* sp.	62.3	4.8		
265	Madison	*Pseudomonas* sp.	36.2	37.4	0.9	4.9
459	Fort Valley	*Bacillus* sp.	58.3	4.5	5.8	11.3
461	Fort Valley	*Bacillus* sp.	86.1	2.0	5.8	0.9

corded as reported previously (see *12*).

To study the total capacity for the breakdown of dieldrin in various soil samples (exclusive of Shell samples that already contained an extremely large amount of dieldrin), a 10-g portion of each sample was first mixed with 100 mg of fine dry glass beads that had previously been coated with 0.1 μmole equivalent (38.1 μg) of C^{14}-dieldrin by using acetone. Soil samples were shaken vigorously to ensure good spreading of dieldrin and were then incubated at 30°C for 30 days for degradation assay. Soil samples treated in this way were extracted twice with 5 ml each of chloroform. The chloroform phase was washed with 10 ml of 0.2 percent trichloroacetic acid (final *p*H, 2).

Whenever the separation of the layers became difficult, the whole system was subjected to brief centrifugation. Sometimes an interphasic layer of organic matter formed. This portion was reextracted with acetone and added to the original chloroform phase. All fractions were radiometrically assayed to find the extent of water-soluble metabolites of dieldrin that are produced by the soil microbes. This treatment with trichloroacetic acid produced a trace of hydrophilic metabolites as an artifact which was compensated for by control experiments.

The chloroform phase was further concentrated by a stream of air to 0.1 ml, and the 10-μl portion of the concentrated material was spotted on a silica-gel (H) thin-layer chromatographic plate. The mobile phase that was adopted to separate various metabolites was a solvent mixture of *n*-hexane and ether at a ratio of 1 : 9, and the system was developed until it was 15 cm from the origin. The resulting chromatogram was subjected to autoradiography with Kodak medical high-contrast x-ray film for 1 month. The portions of silica-gel absorbent that corresponded to the radioactive spots, as judged by the x-ray film, were scraped from the plate, and their radioactivities were determined quantitatively.

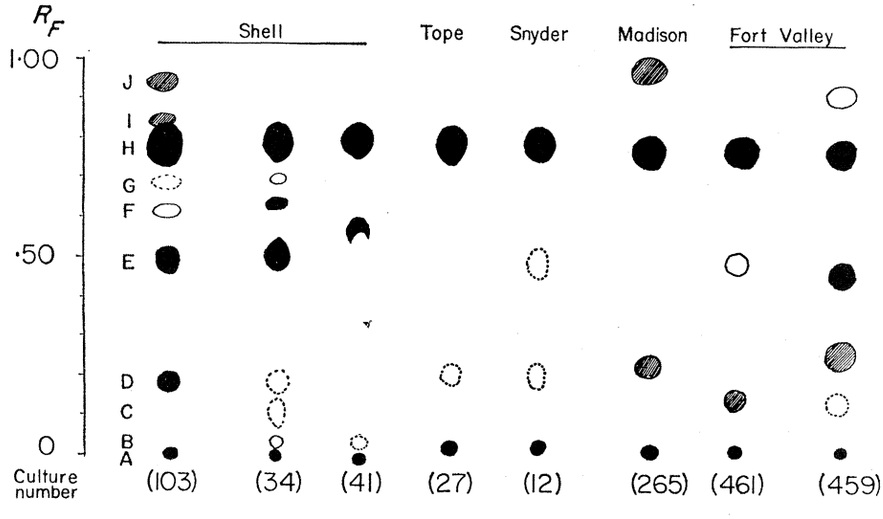

Fig. 1. Radioautographic presentation of thin-layer chromatograms of C^{14}-dieldrin and its metabolites produced by cultures of certain soil microorganisms. Letters A to J designate spots on the chromatogram. Spot H represents dieldrin. Spots showing strongest radioactivity are represented by black; medium radioactivity, shaded; weak radioactivity, open circles with solid line; and the weakest, open circles with dotted line.

As anticipated, the majority of the soil samples tested had no dieldrin-degrading abilities. Exceptions are those from Shell, Snyder, Tope, Fort Valley, and Madison. Besides these 6 soil samples, approximately 30 others from various locations within the continental part of the United States have been screened. The results of extraction and partitioning experiments on soils treated with C^{14}-dieldrin indicated that approximately 1 to 6 percent of added dieldrin was converted to water-soluble metabolites by these soil samples (Table 1). A chromatographic experiment with active soil samples indicated that patterns of metabolite composition in the solvent phases were simple in all cases: that is, only the spot that did not move from the origin (spot A, Fig. 1) and the spot representing dieldrin were predominant, though in some cases other minor spots appeared in the chromatograms.

To study the role of soil microorganisms in the degradation of dieldrin, a total of 577 isolates from soil were screened for dieldrin-degrading activity, although the actual number of species involved was probably much less, since a number of the cultures were no doubt similar (that is, no attempt was made to characterize the isolates unless degradation activity against dieldrin was clearly detected). The majority of the active isolates were found to come from the soils that initially had showed some dieldrin-degrading activity.

Results of assays of the breakdown of dieldrin on several promising soil microbes are summarized in Fig. 1. The thin-layer chromatograms in this figure represent only the chloroform-extractable portion of the sample; percentages of water-soluble metabolites produced from added dieldrin by these active isolates are shown in Table 1. The existence of acidic metabolites of dieldrin among the water-soluble metabolites could easily be demonstrated by collecting the radioactive organic acids with a chromatographic column packed with anion exchanger (Dowex 1 × 8, 100 to 200 mesh) and eluting it with a mixture of strong acid and an organic solvent (con-

centrated HCl, H_2O, and acetone, 1:1:6). A major portion of the water-soluble phase could be recovered by this cleaning method. These metabolites did not move from the origin (spot A) on the same thin-layer chromatographic assay as in Fig. 1, which proves that this portion of the radioactivity is not due to absorbed dieldrin.

None of the dieldrin metabolites found in this work have been identified, except through matching R_F values of various candidate compounds with the use of the same thin-layer chromatographic method. Spot A consisted of materials that did not move by this chromatographic method (that is, very polar compounds) and could contain some amount of acidic metabolites; spot E matched with an authentic reference compound, 6,7-*trans*-dihydroxy-dihydro-aldrin; and spot H matched with dieldrin. The precise mechanisms of the degradation of dieldrin are therefore uncertain at this time. Neither has the actual role of these microorganisms been determined in eliminating this insecticide from the soil.

As shown in Table 1, two out of ten microbial cultures were identified to the species level (cultures 12 and 41 were *Trichoderma viride*). Six of them were found to belong to the genus *Pseudomonas*, and two to the genus *Bacillus*. It is interesting that culture 12 is the same isolate of *T. viride* that was previously reported to have malathion-degradation capacities (*12*). Contrary to this, Chacko *et al.* (*6*) reported this species to be inactive against both DDT and dieldrin. This difference could be due either to differences in testing methods or to differences, at the subspecies level, in the microorganisms themselves. Since the *T. viride* cultures were isolated from two completely different, but dieldrin-treated, soils and since this species is quite versatile in producing numerous variants, one might think that the variants isolated from contaminated soils are indeed very different from those tested by the above workers.

None of the six *Pseudomonas* cultures appeared to belong to the same species. The fact that this type of random screening resulted in the selection of six species of a single genus may indicate the flexibility of the pseudomonads to adapt to situations of high insecticidal pressure.

The major object of this report is to demonstrate, for the first time, the presence in soil of microbes capable of degrading dieldrin. The implications of these findings in the actual ecosystems in our environment may be very interesting. Problems of such an ecological nature, however, are quite complex and will require very careful study.

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14. We thank H. Y. Forsythe, Ohio Agr. Exp. Sta., and Shell Chemical Company for supplying soil samples.

9 January 1967

Field Ion Microscopical Imaging of Biomolecules

Abstract. *Specimen molecules are protected from field desorption by embedding them in a platinum matrix, with the use of electrolytic codeposition on tungsten tips. High-resolution helium ion images are obtained when the biomolecules are exposed during gradual removal of surface layers by controlled field evaporation. Structured images of individual molecules of coenzyme I and vitamin B_{12} are seen.*

The attainment of atomic resolution in images of metal surfaces made with the field ion microscope (*1*) has been an incentive for trying to view organic molecules of biological interest. Unfortunately, phthalocyanine and similar molecules superficially attached to the surface of the specimen tip were found to be removed when a field of about 100 to 180 megavolts per centimeter is applied (*2*), while high-resolution imaging with helium ions requires about 400 Mv/cm. Similar low desorption fields were also found for transfer-RNA and light meromyosin (*3*). An attempt at shadow-casting organic molecules with a refractory metal that would form a complete layer on the surface of the tip and would display the circumference of the removed molecular specimen also failed because the condensed metal layers have a structure too random to be useful (*4*).

A new method was conceived and initially tried out in cooperation with I. R. Miller of the Weizmann Institute. We embed the molecules in an electrolytic metal deposit. During the field evaporation process the embedded molecules appear at the surface and can be imaged. Since the molecule is anchored in the surface and is surrounded by metal atoms on the sides, it will be desorbed at a much higher electric field than if it were simply adsorbed on the surface.

There are two possible ways to include the molecules into the metal matrix. In the first method we tried to adsorb molecules on an interface and subsequently cover the interface with the electrodeposit. If this process is repeated several times, the result is a sandwich-type specimen with layers of adsorbed molecules in the metal. This procedure has the advantage of permitting the use of separate solutions for the organic material and the plating bath, each with a suitable *p*H and the proper polarity of the tip, but it also has some disadvantages. The sample has a tendency to rupture at the intermediate layers during imaging. Irregularities in these layers make image interpretation difficult. The first exploratory investigations (with the plating done by Miller) were made mostly with electrophoretically attached DNA, and subsequent plating with platinum; but no conclusive images could be obtained when the specimens were examined at the Penn State Field Emission Laboratory. In later experiments, the molecules were introduced during the plating process so that, in the end, the molecules were distributed throughout the metal deposit (Fig. 1).

Organic substances present in the plating solution are adsorbed on the