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Fusarium Growth Supported by Hydrocarbons

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ABSTRACT

FLIPPIN, R. S. (Midwest Research Institute, Kansas City, Mo.), CHRISTINE SMITH, AND M. N. MICKELSON. *Fusarium* growth supported by hydrocarbons. Appl. Microbiol. **12:** 93-95. 1964.—In studies of the microflora associated with fuel storage tanks, a *Fusarium* species was isolated from No. 1 diesel fuel. The culture was identified as *F. moniliforme* Sheldon. Attempts were made to cultivate this organism with seven hydrocarbons of 99+ mole per cent purity as the sole carbon source; growth of the fungal culture was supported only by *n*-decane and *n*-dodecane. A spore viability study of *F. moniliforme* in filter-sterilized diesel fuel with no free water showed that viability was retained for 9 months in this environment.

In a study of microorganisms associated with fuel storage tank aqueous phases, we found bacteria, yeasts, and fungi. Numerous microorganisms have been reported which attack hydrocarbons (Beerstecher, 1954). We isolated from diesel fuel a *Fusarium* culture which was of particular interest because its occurrence in hydrocarbon fuels had not been reported.

Fusarium species are widespread in nature, and a number of *Fusarium* species cause plant diseases (Kampmeier, 1959) and fruit and vegetable spoilage (Beraha, Smith, and Wright, 1961); others are prominent in deterioration of paints (Vicklund and Manowitz, 1949) and plasticizers (Klausmeier and Jones, 1961). *Fusarium* species also have been isolated from deteriorated gasoline storage tank linings (Allen and Fore, 1953), but none of the fusaria are reported to be hydrocarbon utilizers. It is important that at least one species of this genus be recognized as capable of fuel hydrocarbon metabolism.

MATERIALS AND METHODS

Source of organism and isolation. Contaminated No. 1 diesel fuel was obtained from a local retail station in the Kansas City area. A pure culture of the fungus was obtained by transferring 1 ml of the contaminated fuel to a sterile petri plate, to which were added 20 ml of sterile mineral salts (Bushnell and Haas, 1941) -agar medium. Plates were incubated for 20 days at 28 C. Only fungal colonies grew on the substrate.

Identification of the organism. The genus of the fungus was identified by C. W. Hesseltine (Northern Regional Research Laboratories); the species, by W. L. Gordon (Canada Department of Agriculture).

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Media. Mineral salts solution (Bushnell and Haas, 1941) or B-H salts plus 1.5% agar (Difco) was used when a hydrocarbon was employed as a carbon source. *F. moniliforme* spores were produced on both a mineral salts-agar medium with diesel fuel and on Mycophil Agar (BBL), the only complete complex medium used in the studies. Plate counts were done on Mycophil Agar.

Growth on 99+ mole per cent purity hydrocarbons. The following 99+ mole per cent purity hydrocarbons were obtained from Phillips Petroleum Co., Bartlesville, Okla.: *n*-hexane, *n*-octane, iso-octane, *n*-nonane, *n*-decane, *n*-dodecane, and *n*-tridecane. Hydrocarbons were Seitz-filtered for sterility. Bushnell-Haas mineral salts medium (10 ml per 16 by 150 mm screw-cap test tube) was dispensed and autoclaved at 15 psi for 15 min. Inoculation of the B-H salts was made by adding to each tube 0.1 ml of a spore suspension which was dispersed in sterile B-H salts solution. Spores for inoculum were harvested from colonial growth of the organism grown in a petri plate on a B-H mineral salts-agar base, to which 0.5 ml of sterile diesel fuel was added. To each inoculated test tube, 10 ml of one of the 99+ mole per cent purity hydrocarbons were added. Incubation was at room temperature (25 to 30 C) for 30 days, after which two serial transfers were made from those tubes exhibiting growth. Visual inspection served as an index of fungal growth.

Spore viability in diesel fuel. The fungal culture was grown on Mycophil Agar until good sporulation occurred. Spores were harvested with a dry loop, and a spore suspension was made in filter-sterilized diesel fuel, containing no free water. A sterile metal 1-quart screw-cap can and a sterile 800-ml clear-glass, screw-cap bottle each received 750 ml of Seitz-filtered No. 1 diesel fuel. Each of these containers was inoculated with a fungal spore dispersion in diesel fuel to give 15 viable spores per ml. Standardization of the inoculum was determined by plating triplicate samples of the inoculated fuel on Mycophil Agar daily for 5 days. Sterile diesel fuel controls in a glass bottle and a metal can were also included in the experiment. Water determinations were made on the inoculated fuel by the Karl Fisher procedure (Ewing, 1954).

Spore counts of the inoculated fuel were made at various intervals over a 10-month period. Inoculated fuel containers were shaken vigorously for 30 sec, and 1-ml amounts of fuel were pipetted into petri plates. Melted Mycophil Agar (20 ml) was poured into the plate, the plate was swirled, and the medium was allowed to harden. Each sample was plated in triplicate. Incubation was at 28 C for 7 days.

Colony counts were used as an index of the viable spores per milliliter of sample.

RESULTS

Identification of the organism. The hydrocarbon-utilizing fungus was identified as *F. moniliforme* Sheldon by W. L. Gordon of the Plant Pathology Laboratory, Canada Department of Agriculture.

Growth on 99+ mole per cent purity hydrocarbons. This culture of *F. moniliforme* produced growth from serial transfers on only two of the seven hydrocarbons evaluated, *n*-decane and *n*-dodecane. Moderate growth formed a fungal mat at the fuel-aqueous interface, and mycelium extended into the fuel but not into the aqueous phase.

Spore viability study. Over a 10-month period, the viability of *F. moniliforme* spores was determined from inoculated No. 1 diesel fuel (Fig. 1). The water contents of inoculated diesel fuel in a metal can and a clear-glass bottle were 500 and 300 ppm, respectively. Under the test conditions, viable spores of *F. moniliforme* Sheldon could be recovered at 9 months from either container. No viable *F. moniliforme* could be recovered from the inoculated containers at 10 months.

DISCUSSION

To our knowledge, this is the first report that growth of a *Fusarium* sp. is supported on a hydrocarbon fuel. One report, however, does state that unidentified species of the genus *Fusarium* were isolated from fueling operations (Hazzard, 1961). No evidence was given in that report that a hydrocarbon fuel was serving as a carbon source for the organism.

The occurrence of the genus *Fusarium* in fuel storage tanks must be rather infrequent in this country. In the examination of over 100 fuel storage tank water-bottoms throughout the midwest area, only one such isolate was found.

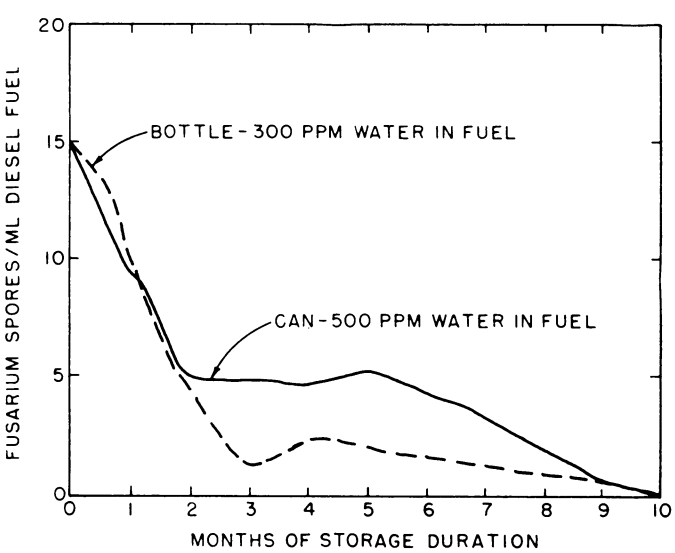

FIG. 1. *Viability of Fusarium moniliforme spores in No. 1 diesel fuel during 10 months of storage.*

Experimental evidence has shown that an active fungal inoculum can be carried by diesel fuel which contains no free water. It is also likely that some bacterial and yeast fuel tank contaminants (those of high cellular lipid content) are also suspended in certain fuels in storage tanks. If this condition exists, the practice of draining water bottoms from fuel storage tanks does not remove the inoculum. Furthermore, without microbiological filtration of fuel, an active inoculum could be transported throughout an entire fuel-handling system.

The culture of *F. moniliforme* that was isolated from diesel fuel does have the ability to use that substrate as a carbon source. However, our attempts were unsuccessful in the adaptation of a stock culture of a closely related organism, *F. moniliforme* USDA 1004.1, to diesel fuel substrate. Failure to adapt the latter culture to diesel substrate would indicate that strain specificity is exhibited in certain strain variations of fusaria.

Due to the presence of additives in diesel fuel, the soluble water (300 to 500 ppm) is high when compared with JP fuel at about 70 to 100 ppm. However, even at a high level of solubilized water, no apparent fungal growth occurred from the spore inoculum in a spore viability study. The indications were that spore death occurred at about the same rate whether they were subjected to total darkness (a simulated field storage condition) or subdued incandescent light (a common laboratory cultural practice). Perhaps the spore respiratory process is reduced to a low level in diesel fuel, and the hydrocarbon acts as a culture preservative such as that obtained by overlaying culture slants with mineral oil (Hartsell, 1956).

In those studies with *F. moniliforme* on 99+ mole per cent hydrocarbons, moderate growth as a fungal mat occurred at the fuel-aqueous interface. Mycelial growth extended into the fuel but not into the aqueous phase. The third serial transfer in *n*-decane and *n*-dodecane showed an increase in the rate of growth as compared with the first inoculum. Growth of the third serial transfer at 2 weeks was comparable with growth of the initial inoculum at 4 weeks.

ADDENDUM

Since the preparation of this paper the following two publications have been called to the authors' attention:

Kester, A. S. 1961. Studies on the oxidation of hydrocarbons by microorganisms. Ph.D. Thesis, University of Texas.

Rogers, M. R., and A. M. Kaplan. 1963. A field survey of the microbiological contamination present in JP-4 fuel and 115/145 avgas in a military fuel distribution system. Microbiological Deterioration Series, Report No. 6, Quartermaster Research and Engineering Center, Pioneer Research Division, Natick, Mass.

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