METHODS and technics for the isolation and identification of bacteriophage of Rhizobium meliloti have been proposed by Laird (2) and Demolon and Dunez (1). These procedures were used as a basis for the bacteriophage studies in the laboratories of the Washington Agricultural Experiment Station, but in the course of this work modifications were introduced by the authors in collaboration with Katznelson. Since the modified method and technic are of a specific nature a detailed report of the entire procedure was considered desirable.

SAMPLING SOILS

Samples for Rhizobium bacteriophage studies should be obtained from the upper 10-inch layer of soil. For the bacteriophage work in the irrigated fields of the Yakima Valley it was not necessary to take soil close to the plant roots exclusively, for in the fields exhibiting alfalfa fatigue the bacteriophage was distributed throughout the upper 10-inch soil layer. However, for 3-year-old or older plantings not exhibiting pronounced fatigue, it is well to obtain the soil samples close to the plant roots and between 2 and 10 inches from the surface. The presence of bacteriophage in the soil under normal conditions should be most pronounced near the heaviest zone of root nodulation. The soil samples should not be allowed to dry out. Isolation work should proceed immediately after the sample is taken or some provision should be made to keep the sample moist until isolation can be started.

Demolon and Dunez (1) claim that freezing causes desiccation and weakening or destruction of bacteriophage in the soil. In view of these findings, it may be advisable to obtain the soil samples in the field before freezing of the soil begins. It should be stated, however, that bacteriophage has been isolated from fields in the Yakima Valley after the surface soil in the field had been exposed to continued freezing temperatures for a period of at least 2 weeks. Either the phage could withstand freezing temperatures, or was present in large enough quantities below the frozen layer to demonstrate its activity in the soil samples taken.

NODULE TISSUES

For the identification of bacteriophage in the nodules, fatigued looking plants are dug up, taking care to leave 1 to 1 1/2 feet of undisturbed soil about the central root to a depth of approximately 16 inches. The fresh, moist soil is carefully pulverized about the plant roots and these are mixed together with a portion of the soil. The roots and nodules are placed in water or moist sand to retain freshness. The nodules are then removed from the plants and tested for the appearance of bacteriophage. Testing is done before the soil has had a chance to dry out and before the plants have wilted, and careful observance of the precautions in making the test are necessary to avoid natural alterations in microbial activity.

ISOLATION OF BACTERIOPHAGE FROM SOIL

Two hundred grams (air-dry basis) of representative soil samples are placed in 500-ml Erlenmeyer flasks with equal amounts of M-5 medium. The flasks are plugged loosely with cotton, allowed to incubate for 48 to 72 hours at 28°C. The culture mixture is then filtered through two thicknesses of medium-fine filter paper or placed in a Buchner funnel and filtered through a fine filter paper, applying what suction is necessary of a suction pump. (If the fluid is not free from sediment, another filtration is necessary.) The fineness of the filter paper and number of filtrations will vary with soil type. The clear fluid is then filtered through sterilized Berkefeld N filter candles and collected in a sterilized test tube attached to the outlet of the filter paper, applying what suction is necessary by use of a suction pump. (If the fluid is not free from sediment, another filtration is necessary.) The filter paper and number of filtrations will vary with soil type. The clear fluid is then filtered through sterilized Berkefeld N filter candles and collected in a sterilized test tube attached to the outlet of the Buchner funnel and filtered through a fine filter paper, applying what suction is necessary by use of a suction pump. (If the fluid is not free from sediment, another filtration is necessary.) The clear fluid is then filtered through sterilized Berkefeld N filter candles and collected in a sterilized test tube attached to the outlet of the Buchner funnel and filtered through a fine filter paper, applying what suction is necessary by use of a suction pump. (If the fluid is not free from sediment, another filtration is necessary.)