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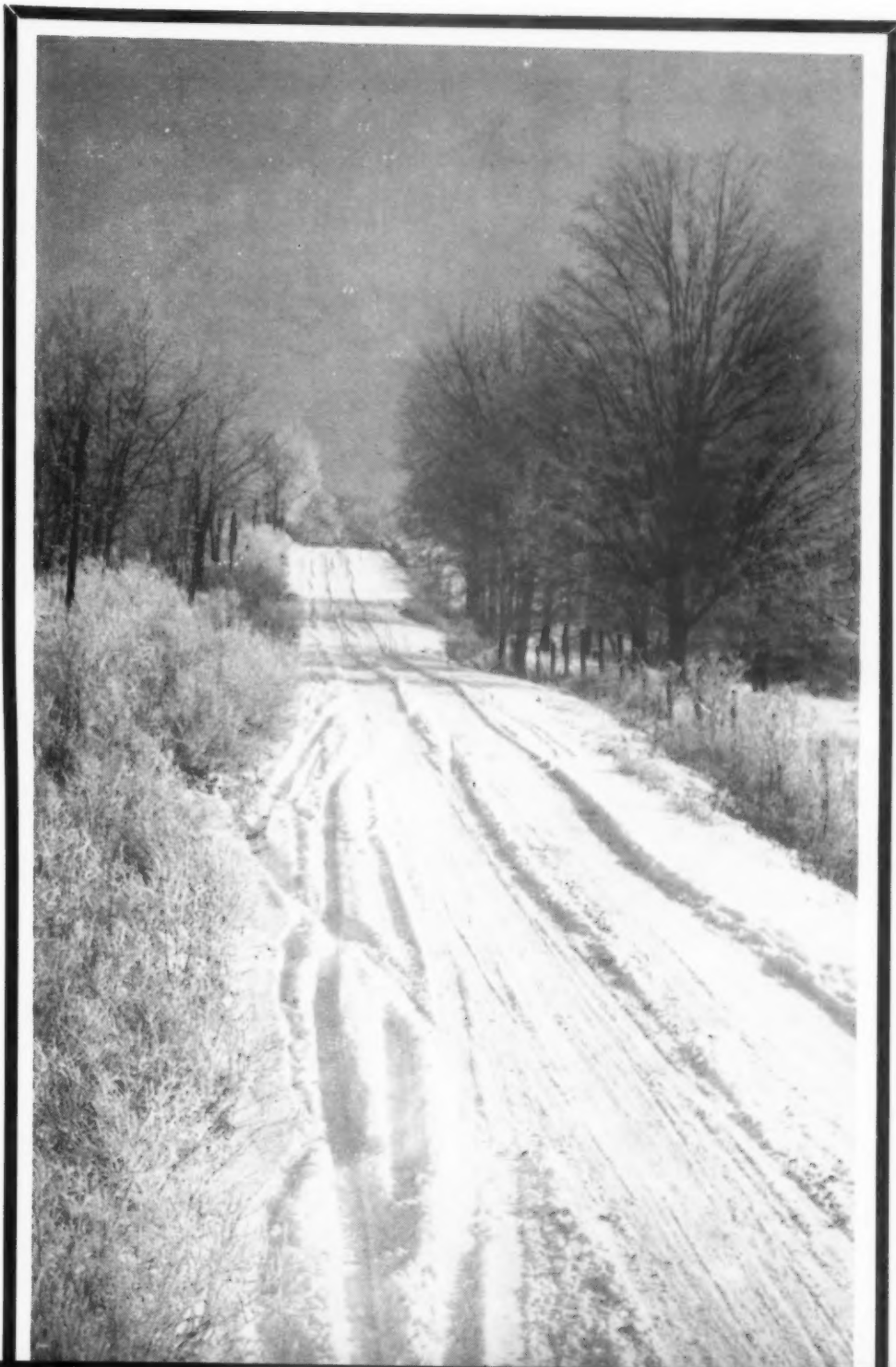
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A Simple Field Test For American Foulbrood

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IN the course of a study on the physiology of *Bacillus larvae*, the cause of American foulbrood of honeybees, several unique enzyme properties of this organism were revealed. Certain of these enzymes suggested that they might be made the basis of a field test for American foulbrood, a test which has long been needed. In the consideration of such a test the criterion of reliability was of paramount importance, with other features, such as time required to run, cost and availability of equipment, simplicity of performance, and ease of interpreting the results, as important secondary considerations.

The enzyme causing the liquefaction of milk casein or curd from either reconstituted or skim milk was found to be best suited as the basis for a test for American foulbrood. It is produced by *Bacillus larvae* when the spores are formed, and is thus abundant in "ropy" larvae, persisting over in the scales for years if kept under ordinary conditions.

The test, as first set up, consisted in dropping the suspected material into a vial containing about 6 drops of milk. The vial was kept at 165° F. in a waterbath during the test. With American foulbrood material the milk curdled within 1 minute. Almost immediately after curdling the liquefying enzyme began to hydrolyze the curd, and this reaction was usually complete in 15 minutes. Such a test was specific for American foulbrood.

During demonstration of the test in the field it was found that addition of water to the milk made the test easier to follow. Subsequent experiments showed that, by adjusting the proportions of reconstituted or skim milk and water, the test could be run at ordinary temperatures.

The following test, under the condition described below, has never failed to detect American foulbrood disease, nor have any "false positives" been obtained with any non-American-foulbrood diseased larvae, pollen,

bees, or other material found in the hive. The initial cost for the equipment required is only a few cents, while the materials used for over 100 diagnoses will cost less than 1 cent. The diagnosis can be made immediately in the apiary, or the suspected material may be run later in the home, office, or laboratory.

This test is intended for use of inspectors or operators having frequent need for an American foulbrood test. An alternate method is included for use by the beekeeper who might want to perform the test only occasionally. Materials needed are skim-milk powder, distilled or tap water, a 1-gram homeopathic vial, and a medicine dropper.

Reconstituted milk is prepared from the milk powder by adding 4 level tablespoons to a quart of water. It is recommended that the milk be freshly made up the day the test is run, to avoid souring. Various types of water have been used with no unfavorable results. However, if there is any doubt as to the water, the test can be run with a distilled-water check and the results compared. Best results are obtained if the water is warm but not uncomfortably hot. The test will work, though, at a temperature around 50° F. (10° C.). Under such conditions somewhat slower clearing can be expected. If the water is cold, it should be warmed. This can easily be done by holding the vial in the hand, if only a few samples are to be run.

To run the test, place the sample in the vial and add 20 drops of warm water (about ¼ of a vial) and shake gently. Then add 10 drops of the powdered-milk solution, and again shake gently. If less than entire scale is available, add 20 drops of water as before, but reduce the number of drops of milk proportionately. There may or may not be a fine curd after 5 minutes, but this is not a significant part of the test. The test is positive if the milky suspension clears, which

usually occurs within 15 minutes, leaving a transparent, pale-yellow liquid. Some times the clearing is so rapid that a test is definitely positive after 5 minutes. The difference between a liquefied positive test and a negative or check test is very striking if the vials are held to the light. Until one is familiar with the test as obtained with American foulbrood material, it is advisable to have a check vial, with only water and milk suspension for comparison. With non-American foulbrood scales the liquid may sometimes become somewhat discolored, but the suspension remains cloudy during the 15 minutes of the test, and the test is considered as negative.

In case only one or a few tests are to be run, it is not essential that reconstituted milk be prepared. Add 20 drops of warm water as before and shake; then add 5 drops of whole milk (skim preferred), instead of the reconstituted, shake, and read as before. If less than an entire scale is available, reduce the amount of milk proportionately. For example, if half a scale is tested, add 2 to 3 drops of milk.

The test possesses a certain amount of flexibility; that is, it need not be performed in the field, exactly as described above. If for some reason the inspector cannot or does not choose to run the test immediately, the samples can be placed in the vials and tested later at the laboratory or at home. In fact, successful tests have been run with match-stick samples of ropy material, as are frequently submitted for diagnosis. In these cases only 5 drops of the reconstituted milk were used, since the ropy material is only partly removed by this technique.

As a matter of good practice, care should be exercised in disposing of the material in the vial after the test is run. The vials should be washed

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conjunction with the Ohio Farm and Home Week, February 5, 6 and 7, the Ohio State University, Columbus, Ohio. The Beekeeping Section will cover the three days with the banquet scheduled for the evening of February 6th. Out-of-state and Ohio speakers will furnish talent to cover post-war problems confronting the Beekeeping Industry.

A Simple Field Test For American Foulbrood

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clean, and boiled 20 minutes in water before re-use.

The cooperation of beekeepers and inspectors in submitting reports of any interference with the test through water source, drug or chemical treatment, and the like, will be appreciated. For example, it has been noted that scales treated with formaldehyde fumes give a negative reaction. (1) Similarly, the test is retarded when scales are exposed to P-dichlorobenzene (PDB) vapors. On the other hand, sulfa drugs added to scales had no effect. Reports of any negative tests occurring with larvae in the very early stages of American foulbrood are also desired. In any doubtful cases the Division of Bee Culture Investigations of the Bureau of Entomology and Plant Quarantine will gladly make a laboratory analysis of the material.

(1) A. G. Lochhead, personal communication.

Wood Mint

(Continued from page 17)

would probably result in cutting of the plants when in full bloom and thus greatly reduce the flowering time.

In the wild state its habit of growth is such that it can never be an important source of honey. As far as the writer has been able to observe it does not grow in mass but always in small scattered clumps. As a garden flower the quantity available would offer little help to the beekeeper as we find little hope of adding substantial bee pasture from this source. Its attraction for the bees is such that it is hard to understand how it could have been so completely overlooked.