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# STAINING METHODS FOR DETECTING WEEVIL INFESTATION IN GRAIN

By J. C. Frankenfeld
Division of Cereal and Forage Insect Investigations

The eggs and immature stages of the rice weevil (Sitophilus oryza (L.) and the granary weevil (S. granarius (L. )), hidden within the kernels of wheat, corn, or other grain, are invisible to the naked eye and cannot be detected by ordinary inspection methods. Grains that grade No. 1 may actually have a high percentage of infested kernels. If this grain is used for milling, the different stages of the weevils, together with the cast skins and excrement, will be ground up in the milling process and the finished flour or meal will thus be contaminated with the fragments. In order that the miller may produce a pure product he must know the condition of the grain he is grinding. So far as the miller is concerned, it matters little whether the infestation in the grain is alive or dead, or what stages are present, since any stages of the weevils from egg to adult, whether living or dead, will contaminate the finished product. A miller may therefore buy a No. 1 grade grain, which apparently is free of infestation because of fumigation and cleaning methods applied prior to its arrival at his mill, but which may, nevertheless, be heavily infested with immature stages of weevil hidden within the kernels.

A simple, practical, and inexpensive method has been devised by the author for detecting this hidden infestation in grain. It consists of treating samples of the grain with a dye which stains the egg-plugs and weevil punctures. In depositing her egg the female weevil first drills a tiny cavity into the surface of the grain kernel. After depositing her egg in this cavity she seals the opening with a gelatinous secretion, which hardens and serves as a protective plug. This plug remains on the grain throughout the development of the immature stages, and, unless removed by the emerging adult weevil, will remain on the infested grain indefinitely. Therefore, no matter what stages of the weevils may be present, any infested kernel can be easily detected by the use of a dye that has an affinity for the egg-plugs.

There are two dyes which may be used in testing for weevil infestation in grain. Because of its decided advantages, the author prefers the acid fuchsin dye. It is prepared with the following ingredients:

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| Acid fuchsin | 0.5 gm. |
| Glacial acetic acid | 50.0 cc. |
| Distilled water | 950.0 cc. |

Mix the distilled water and glacial acetic acid and then add the acid fuchsin which dissolves readily in this solution. Care should be taken

not to confuse acid fuchsin with basic fuchsin, which is entirely different in its action and is not suitable for detecting weevil infestation in grain. Acid fuchsin and glacial acetic acid are relatively inexpensive and may be obtained from any drug or chemical supply house.

It is not necessary to prepare fresh solutions of acid fuchsin. Any desired quantity may be prepared at one time, stored, and used as needed without loss in efficiency. The same solution may also be used a number of times without losing its efficiency. If after prolonged usage the solution tends to become murky it should be discarded.

The samples of grain to be tested should be placed in a suitable container, preferably a shallow dish large enough to adequately hold the sample and solution. Enough of the solution should be used to cover the kernels completely. The grain is left in this solution from 2 to 5 minutes. If left in the solution for a longer period the kernels will absorb enough of the solution to color them slightly, thus making it more difficult to locate the egg-plugs. If the grain is permitted to soak in warm water for 5 minutes before being treated, less staining of the kernels results without impairing the staining of the egg-plug. The water should be poured off before the fuchsin solution is added. After the grain has been immersed in the fuchsin dye from 2 to 5 minutes pour off the dye and wash the grain in tap water until all the excess dye has been removed. It may then be examined under water or drained and spread on a glass plate or similar surface for examination.

The acid fuchsin stains the gelatinous egg-plugs a deep cherry red, whereas feeding punctures and mechanical injury are stained a light pink. The egg-plugs (fig.1) are about the size of an ordinary pin prick and are readily seen with the naked eye, but the use of a reading glass is helpful in finding them.

Besides staining the weevil egg-plugs, this dye will also stain weevil feeding injury, as well as entrance and exit holes of the lesser grain borer. These are stained a lighter color, however, and can therefore be easily differentiated from weevil egg-plugs. Mechanical injuries, as a result of threshing and subsequent handling of the grain, will also be stained. However, they may be easily distinguished from the egg-plugs by their shape, size, and lighter color. Insect feeding injuries are round and smooth in outline, while mechanical injuries are very irregular. Grain that has passed through cleaning machinery, such as scourers and disk separators, prior to milling, frequently bears injuries that greatly resemble the feeding injuries of weevils and grain borers. It is practically impossible to differentiate these two types of injury. However, the miller who wants to know whether the grain contains weevil infestation can find this out by using the stain on samples of the grain before it is run through the cleaning machinery, thus avoiding any confusion in distinguishing weevil egg-plugs and weevil or grain borer feeding injuries from mechanical injuries.

The proportion of weevil egg-plugs to feeding punctures will depend largely upon (1) the number of adults to which the grain has been exposed; and (2) the condition of the grain, particularly as to moisture content.

Apparently the adult weevils feed on the endosperm of the grain kernel in the process of drilling the cavities in which they lay their eggs. Thus, unless the weevils are disturbed, eggs are usually deposited in all cavities that the adults drill into the kernels. If the populations are very large many punctures that do not contain eggs may be found. This is because the female weevil was disturbed while drilling the egg cavity and did not return to deposit the egg. The author does not consider this a significant factor, however, for in all such cases encountered there were one or more egg-plugs in addition to the egg-free punctures.

If the moisture content of the grain is comparatively low, that is 11 percent or less, the female weevil may drill a cavity but, finding conditions unsuitable, will not deposit an egg. Such grain will generally have a comparatively high percentage of egg-free punctures. In numerous dissections of kernels that had been treated with this dye to locate the egg-plugs, weevil eggs were found under every plug. The female weevil does not seal the cavity in the kernel unless she has deposited an egg. By actual count the total hatch is from 85 to 90 percent of all eggs laid. With moisture and temperature conditions remaining favorable, all larvae that hatch will complete their development.

Since this dye stains the weevil egg-plug, it is suitable for determining weevil infestation in wheat, corn, and sorghum grains. Fifteen varieties of hard and soft wheat have been tested, and on all of them the egg-plugs could be readily detected. Polished rice takes up too much stain to reveal egg-puncture plugs. The stain has not been tried on other grains, and its effect on the eggs of weevils that glue their eggs to the surfaces of dry beans and peas has not been determined.

Weevil eggs may be readily distinguished from the eggs of other species of grain-infesting insects, because all other species lay their eggs loose among the kernels, in larger feeding cavities in the kernels or in floury material mixed with the grain.

Correction Slip

U. S. Bureau of Entomology and Plant Quarantine ET-256

The fact that this is a report of a study made under the Research and Marketing Act of 1946 was inadvertently omitted.

Page 3, last paragraph--In place of first 8 lines, including that which ends "This dye, commonly used as a," substitute the following:

> The other preparation that may be used is an iodine solution which is prepared by diluting 20 ml. of Lugol's solution, obtainable from any drug or chemical supply house, with water to make a total volume of 900 ml. This preparation, commonly used as a ----

In general, the method used is the same as for the fuchsin dye except that a 10-minute period is required to obtain maximum staining. An iodine solution should always be fresh when used because it quickly loses its efficiency upon standing. It is also important to examine the grain soon after staining because the color gradually decreases in intensity when exposed to the air.

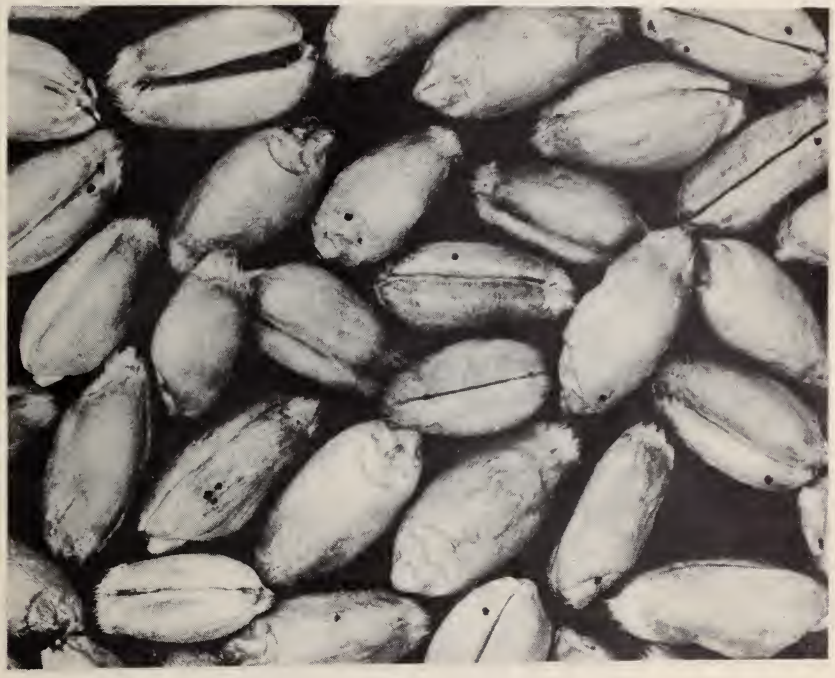

Figure 1.--Wheat kernels showing egg-plugs of the granary weevil stained with acid fuchsin.