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REARING BOLL WEEVILS ON AN ARTIFICIAL DIET

J. R. Brazzel, T. B. Davich and Klaus Raven*

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SUMMARY

A technique was developed for rearing large numbers of boll weevils from egg to adult in lots of 50 on an artificial diet under close confinement. The addition of a mixture of sorbic acid and methyl para-hydroxybenzoate (methyl paraben) to the diet eliminated the necessity of asceptic procedures. About 70 percent of the eggs transferred to the diet developed into adults in 14 to 18 days. Weevils reared on this media were uniform in size and averaged about 12 milligrams in weight.

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Introduction

Boll weevils for use in laboratory toxicity studies were obtained in the past by collecting adults from infested cotton fields or by picking infested squares from the field and holding them in the laboratory until the weevils emerged. Recent work reported by Rainwater & Gaines (1951), Reiser et. al (1953) and other workers showed that considerable variation existed among weevils collected from different sources and at different times of the year. With field-collected insects, boll weevil studies were restricted to the period of the year when the insect was available in required abundance in the field.

The occurrence of resistance to chlorinated hydrocarbon insecticides in the boll weevil in Louisiana (Roussel & Clower, 1957) and later in Texas (Walker et. al, 1956) resulted in an intensified research program along biological, ecological, physiological and toxicological lines. These studies required a constant supply of standardized cultures of the boll weevil. Vanderzant & Davich (1958) reported a semi-synthetic larval diet and technique for rearing weevils from egg to adult in individual containers under asceptic conditions. Earle (1959) modified this larval diet by substituting acetone-extracted cotton squares for the soybean protein and adding a mixture of sorbic acid and methyl paraben to reduce interference with growth and development by contaminating microorganisms. With these modifications, he was able to rear weevils in individual shell vials under non-aseptic conditions. Labor costs were excessive for rearing the large number of weevils necessary for research work by the procedures cited above.

The technique for mass rearing weevils on a larval diet in groups of 50 individuals per rearing container under non-aseptic conditions is reported here.

*Respectively, associate professor, Department of Entomology, Texas Agricultural Experiment Station; entomologist, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture; and graduate student, Department of Entomology.

Experimental Procedure

Several chemicals were tested to determine their effectiveness as antimicrobial agents. The materials included Roccal, sorbic acid, sodium benzoate and methyl, ethyl, propyl and butyl parabens. These chemicals were tested as surface-sterilizing agents by applying them to the surface of the sterilized diet and by incorporating them into the unsterilized diet. Petri dishes were used as rearing containers. The effect of the various inhibitors on the boll weevils was tested by rearing larvae aseptically in media which contained various concentrations of the chemicals tested. Data were obtained on toxic effects, weevil size and duration of developmental period.

Eggs were transferred to the medium in a liquid suspension and by means of a brush. The liquid transfer of eggs consisted of pipetting approximately 50 eggs randomly over the smooth surface of the medium in a solution of 3,000 ppm Roccal. The brush technique involved transfer of the eggs from the same concentration of Roccal solution into individual holes made in the diet. The larval feeding holes were spaced at approximately 1 centimeter intervals and were made with a device which allowed punching of all holes in one dish of diet in one operation.

Eggs were obtained by dissecting cotton squares that had been exposed to weevils for 24 hours in an oviposition cage.

Results

None of the chemical inhibitors tested by application to the surface of the medium reduced the growth of microorganisms satisfactorily. The transfer of eggs on the surface of the medium in Roccal solution also was unsatisfactory because of the failure of the larvae to enter the diet and because of excessive cannibalism.

Dosage mortality lines were obtained for all the inhibitors incorporated in the diet except Roccal. Results indicated that less than 5 percent mortality resulted from exposure of the larvae to 0.2 percent sorbic acid and 0.15 percent methyl paraben. The other three parabens tested were considerably more toxic. The toxicity of sodium benzoate to the weevil was lower, but inhibition of microorganisms was inferior to the other materials. Sorbic acid or methyl paraben used alone at dosages that would kill 5 percent of the larvae were not effective inhibitors; however, when used in combination, these two materials controlled the growth of microorganisms satisfactorily.

The combination of sorbic acid at 0.2 percent and methyl paraben at 0.15 percent included in the diet caused less than 5 percent weight reduction in the weevils and had no measurable effect on the duration of the developmental stages. These inhibitors did not reduce significantly the percentage weevil eggs which hatched. A comparison of these inhibitors in the soybean protein diet (Vanderzant, 1958) with the cotton square protein diet (Earle, 1959) indicated a higher level of microbial inhibition when the protein source was acetone-extracted cotton squares. Similar results were obtained when acetone-extracted cotton leaves were substituted for a portion of the soybean protein, except that the weevils weighed approximately 1 milligram less than in case of individuals reared on the extracted-square diet.

In addition to incorporation of inhibitors in the diet, several other practices were followed to decrease the incidence of contamination. Eggs were surface sterilized with a 3,000 ppm solution of Roccal immediately before transfer to the diet. Eggs could be transferred directly from the Roccal solution to the diet. All petri plates were sterilized in an oven for at least 1 hour at 180° C.

The depth of the diet in the petri plates and the moisture content were critical factors in reducing the growth of microorganisms. The diet was poured to a depth of about 6 millimeters in the petri plates. This depth was sufficient to prevent the larvae from breaking out of the feeding cell and wandering about over the surface of the diet and allowed the diet to dry sufficiently to arrest local spots of contamination. The drying of the diet was done by covering dishes immediately after inoculation with cardboard dessicator tops. After 24 hours these tops were replaced with the glass petri plate cover.

Most of the weevils emerged in 14 to 18 days after the eggs were placed on the diet. With the media poured to a depth of 6 millimeters, the adults were able to emerge from the pupal cell unassisted. Adults were obtained from about 70 percent of the eggs implanted in the diet.

Detailed instructions for mixing and the ingredients necessary for adequate boll weevil larval diet to pour 12 petri plates follow:

1. Weigh the following ingredients into container 1: 20.0 gm of square meal or leaf meal and 25.0 gm of soybean protein.

2. Weigh the following ingredients into container 2: 25.0 gm sucrose, 0.15 gm L cysteine HCL, 0.5 gm Glycine, 0.25 gm Cholesterol, 5.0 gm Wesson Salts, 2.5 gm Sodium Algenate; and 5.0 gm Brewers yeast.

3. Weigh 15.0 gm agar into container 3.

4. Heat 430 ml of distilled H_2O to boiling. While the water is heating, the following liquid ingredients are measured into the mixing bowl of a 1 quart Waring blender: 10.0 ml of inhibitor solution. (The inhibitor solution is prepared by mixing 20.0 gm of sorbic acid and 15.0 gm of methyl paraben in 170.0 ml of ethanol.); 14.0 ml of KOH (10 percent); 5.0 ml of vitamin solution (constituents given by Vanderzant, et. al , 1957); and 2.5 ml of Mazola oil.

5. After the water begins to boil, add the agar in container 3 and stir until dissolved. Pour the dissolved agar solution into the mixer and mix at low speed as the ingredients in container 2 are added slowly. After this solution is mixed thoroughly, increase the mixer speed and add the content of container 1 slowly. When the contents have been mixed thoroughly, pour the media into sterilized petri plates. This amount of media is sufficient to pour 12 plates.

After the media has solidified, holes are made in the top for the eggs. Eggs are sterilized in 3,000 ppm solution of Roccal and placed individually in the prepared holes in the media with a camel's hair brush. No further aseptic precautions are necessary during the transfer of eggs. The rearing containers were covered with a dessicator top of cardboard for 24 hours and replaced with the glass top.

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