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SEROLOGICAL COMPARISON OF THREE SPECIES
OF GRAIN INFESTING WEEVILS
(CURCULIONIDAE: SITOPHILUS)

by 866

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B.S., Kansas State University, 1963

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

Kansas State University
Manhattan, Kansas

1965

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INTRODUCTION

A knowledge of the biochemical similarities and differences of the members of the rice-weevil complex may be of value both in the determination of species and in evaluating population divergences. Confusion exists in the literature as to the correct naming of two of the species of Sitophilus.

Weevils of the rice-weevil complex are not identifiable by external characters and taxonomists are not in agreement on the usefulness of the genitalia. Yet, size, behavior and inability to produce viable eggs after cross-mating point to distinct species.

This study is to determine if biochemical differences exist among common grain infesting weevils and if so can these differences be determined using serological techniques. In the search for differences the precipitin test was used. This procedure included turbidometric measurements and reaction of antigen and antibody in agar media.

The three species used were Sitophilus oryzae (L.), Sitophilus zeamais (Mot.) and Sitophilus granarius (L.). Significant morphological differences and biological variations have been noted by Soderstrom and Wilbur (personal communication).

This study was designed to give a basis for later studies of population divergences among these members of the genus Sitophilus.

REVIEW OF THE LITERATURE

Precipitin Tests and Antisera Preparation

The precipitin reaction was developed by Kraus in 1897. When first reported, it was claimed to be absolutely specific, i.e., each antiserum reacted only with the particular kind of antigen used in its formation. The early impression that the precipitin reaction was absolutely specific was disproved by Nuttal (1904) who later proposed the principle of quantitative specificity. This principle implies that each antiserum reacts most strongly with the particular antigens used in its formation (homologous reaction) and less strongly with other closely related antigens (heterologous reaction) under comparable conditions. This principle was given impetus with Bordet's (1899) work on anti-fowl serum.

Ascoli in 1902 developed the ring test which displaced the mixing method used by earlier workers. In the ring test, antiserum was layered beneath the antigen in a small tube and the precipitate formed at the interface or the antigen in some cases was layered above the antiserum. Earlier "ring tests" could be taken only as approximations because the homologous and heterologous reactions were not treated in comparable ways.

Recent modifications of the precipitin test involve the reaction of antigen and antibody in agar media. Cuchterlony (1949) developed the plate test in which antigen and antiserum diffuse out from wells in the agar media and may form several zones or bands of precipitate in the agar media. A second antiserum-agar test, the tube test developed by Cudin (1952), involves the mixing of antiserum and agar in slender tubes and allowing the antigen (overlay) to diffuse through the gel column. This test is more sensitive, producing more bands than the plate test, but is limited in the respect that only one antigen

can be compared whereas several antigens can be cross-compared in the plate test.

In systematic serology, more exact results are usually obtained by the turbidimetric measurement of turbidity resulting from mixing of antigen and antibody than from the ring or interfacial test. The fundamental principle is again that of quantitative specificity. This principle guarantees that every antigen, whether in a mixture or not, for which there are any antibodies in the antiserum used, is present in adequate amounts to satisfy completely the combining capacities of those antibodies (Boyden, 1963). Turbidimetric measurement procedures were used by Boyden (1942), Boyden and DeFalco (1943), Boyden and Gemeroy (1950), Gemeroy (1943), Leone (1949, 1950), Moody (1958), Moody, Cochrane and Drugg (1949), Moody and Doniger (1956), Stallcup (1954), Wenyss (1953) and others.

Leone (1952) and Proom (1943) showed that the first antibodies are more specific than those resulting from subsequent injections. Leone also found evidence that the specificity of an antiserum may depend on the physiological state of the animal. Wolfe (1935, 1936) reported that a few small injections produced more specific antiserum than several large injections.

Proom (1943), in his studies on route injection, obtained the highest-titered antisera from intramuscular and subcutaneous injections; much lower titers were obtained from using intraperitoneal and intravenous routes. Landsteiner (1947) found that the individual variation in animals is an important factor since some fail to respond to repeated dosages. Boyden (1926) found variation in antibody responses in individuals of the same species, using the same antigen and injection procedures.

Boyden and DeFalco (1943) stated that the Libby photronreflectometer appeared to be an instrument by which species, and sometimes types within

species might easily and consistently be distinguished.

Experiments by Brown and Heffron (1928), Martin and Cotner (1934), and Cumley (1940) have shown high correlation between the precipitin reactions of extracts of insect bodies and their present systematic positions. The development of systematic serology was reviewed by Boyden (1953) and the entomological applications thereof reviewed by West (1950), Downe and West (1954) and Downe (1957).

Leone (1947a, b) evaluated five families of the order Orthoptera and found correlation between taxonomic and phylogenetic studies. This indicated that there may be some correlation between the time of origin and the degree of similarity in the antigenic constituents of insects.

Downe (1962) used precipitin tests on the cuticular extracts of house fly larvae and showed the presence of several antigenic protein components. He also found that a considerable degree of serological specificity was exhibited in precipitin tests by the cuticular proteins of seven species representing four orders of insects.

Lawlor (1949) combined complement fixation and precipitin test techniques for a comparison of five species of mosquitoes. West et. al. (1950) used the precipitin test to study the systematics of Neodiprion sawflies.

Weitz (1956) stated that antisera stored in the frozen state showed no change in characteristics but recommended freeze-drying if storage was extended. Antisera stored at 5° C. for several weeks to one year usually showed a significant loss in titer (Brooke and Proske, 1945; Eligh, 1952).

Rice-weevil

Rice-weevils were first reported by Carolus Linnaeus and named Curculio oryzae L. (Linnaeus, 1749-69). The confusion brought about by his size

description has led to the description of several species, varieties, strains and populations.

Kuschel (1961) in his study of synonymy in the Sitophilus oryzae complex gave a chronological list of names in question in the complex. Kuschel separated the two species, Sitophilus oryzae (L.) and S. zeamais Mot., by the aedeagus and the microsculpture of the prothorax and elytra. He thought that separation of species can be achieved with certainty only by genitalia characteristics. Kuschel considered that the microsculpture of the thorax was a difficult and uncertain character. The most reliable and easiest character to use is the male aedeagal character given by Kuschel; oryzae, upper surface of the aedeagus evenly convex; zeamais, upper surface of the aedeagus flattened and with two distinct longitudinal impressions.

Kono (1955) demonstrated intersterility in a cross of a Nepali small form with the large form from Japan. Crosses between the Nepalese form and small forms from both Japan and Australia were fertile. Soderstrom (1962) found differences between two populations of lesser rice weevils in their ability to transfer sperm to the larger rice weevil.

Many characteristics have been used for the identification of the two rice weevil species. Size was used by Motschulsky (1855), Sasaki (1899), Takahashi (1928) and others. Color differences were used by Richards (1943), the small weevil being brown and the large weevil black. Kono (1955) considered the sculpturing of the pronotum to be a reliable character.

Biological variation studies by Soderstrom and Wilbur (personal communication) have indicated the following:

1. The Kansas population, S. oryzae (L.) differed significantly in the production of progeny from the Arkansas population, S. zeamais Mots. when reared in Ponce wheat and Martin sorghum.

2. The average developmental time for Arkansas zeamais was 39.1 days and 41.7 days for Kansas oryzae when reared on various grains.

3. In population competition studies, the Arkansas population of zeamais increased faster than the Kansas population of oryzae.

4. In a study of frass ejection from Ponca wheat kernels by larvae, the amount of frass ejected from the kernels per larva was 42 micrograms for Kansas oryzae, and 320 micrograms for the Arkansas zeamais.

Granary Weevil

The granary weevil, Sitophilus granarius (L.) was first recognized as a distinct species by Linnaeus in 1758. It is easily distinguishable from the closely related and more destructive rice weevils. The granary weevil has lost all but a vestige of its wings, while the closely related rice weevils retain wings and some are good fliers.

The granary weevil is a polished, chestnut brown to blackish beetle similar in size and shape to the rice weevil. The distinguishing character that separates the granary from rice weevils is the shape of the small pits on the prothorax (dorsal) and the presence or absence of wing spots on the elytra: in the rice weevil, the pits are round and the wing spots present whereas the pits are oval and the wing spots absent in the granary weevil.

Descriptions of the granary weevil life cycle have been reported in the literature by Back and Cotton (1922), Nakayama (1926) and Kirkpatrick (1962).

MATERIALS, METHODS AND EQUIPMENT

Source of Insects

Weevils used in these experiments were obtained from stock cultures at Kansas State University, and were originally obtained as follows:

1. The lesser rice weevil population, Sitophilus oryzae (L.) and the granary weevil population, Sitophilus granarius (L.) were from infestations in Kansas farm stored grain.
2. The rice weevil population, Sitophilus zeamais Mot., was obtained by Paul Boles from stored corn near Stuttgart, Arkansas in 1955.

Culture Media and Preparation

The grain used was Ponca variety of hard red winter wheat which was held in cold storage at Kansas State University until ready for use.

The grain was cleaned using a Bates Laboratory Aspirator adjusted to remove dockage, stored in sealed containers and put into a deep freezer for seven days at approximately 0° F. to destroy insect infestations. After this period the grain was removed and brought to room temperature, its moisture content was determined by using the Steinlite moisture tester (Model 400G) and the Motomco moisture tester (Model 919). If grain moisture level was too low, water was added to obtain the desired moisture level, and if too high, the grain was dried on a table with a current of air being supplied by an electric fan.

The calculation procedure used to get the proper moisture level was the standard procedure used in the Stored Grain Insect Laboratory at Kansas State University. The procedure is as follows:

$$\frac{100 - \text{the present \% water content}}{100 - \text{the desired \% water content}}$$

The first digit of the quotient, which was always one, was dropped, leaving the remainder of the quotient as a multiple factor that was multiplied by the amount in grams of wheat to be tempered. The product was the amount of distilled water that should be added to the grain to bring the moisture content to the desired moisture level. The correct amount of water was added to the barrel of grain and the lid sealed. Several days later the moisture content was checked after the wheat had been mixed several times by rolling on a barrel roller. These procedures were repeated when the moisture content did not meet the requirements of 13.5 percent \pm 0.5 percent.

Rearing Methods

The standard rearing procedures were those used in the Stored Grain Insect Laboratory at Kansas State University. These procedures have been reported by Soderstrom (1962) and Kirkpatrick (1962). Temperatures were maintained at approximately 80° F. and relative humidity at approximately 70%.

Procedure for Obtaining Test Insects

In preparing the weevil stock cultures, approximately 500 grams of insect free, 13.5 percent moisture wheat was placed in each one quart, wide mouth jar in which the metal top had been replaced with a 40-mesh screen. Kelthane treated filter paper was placed under the screen to keep the cultures mite free.

Approximately 200 fifteen day-old unsexed adult weevils were placed in each jar for seven days to obtain eggs. At the end of the oviposition period, the parents were removed from the wheat and the wheat returned to the jar. All cultures were returned to the rearing room and maintained under normal rearing conditions until enough adults could be obtained for extraction.

Preparation of Antigens

The preparation of a cell-free extract used as an antigen was patterned on the procedure described by Leone (1947). Weevil adults were starved for 48 hours to eliminate food from the gut, thereby reducing the possibility of contamination from the gut contents. The weevils were killed by freezing and the extraneous material was removed by washing the insects in several changes of distilled water and in 0.167M sodium sulfate solution, after which they were dried by gently rolling them between paper towels. The adults were then macerated using a mortar and pestle with the addition of buffered physiological saline during maceration as used by Evans (1922). The volume of saline added was equal to four times the weight of unmacerated adults: for example, for each gram of insect used, the extraction process required 4 cc. of physiological saline. The saline-insect mixture was then placed in bottles and refrigerated at 5° C. (46° F.) for 48 hours. At the end of this period the extraction mixture was filtered through a Buchner funnel using negative pressure from a water faucet or a vacuum pump. The filtrate was then sterilized by passage through a Seitz filter and was stored in 20 cc. serum vials.

Rabbits

Young adult rabbits (2 to 3 kg.) were used for antisera production. They were caged separately in the laboratory and observed closely during the period of experimentation for signs of unfavorable reaction to the inoculated material. Rabbits were selected over guinea pigs because of their higher antisera production and the ease with which they can be maintained.

Preparation of Antisera

The procedures used for antisera preparation were patterned after those of Leone (1947) and Edman (1964). Antisera were prepared against Sitophilus oryzae (L.), S. zeamais Mot. and S. granarius (L.). One rabbit was used for the production of antisera against any one antigen. The antigen injection series consisted of an initial dose of 1 cc. given intravenously and followed on alternate days with 1 cc. injections given subcutaneously. Following a rest period of 10 days from the last injection, trial bleedings were performed. Booster injections were given on alternate days after each bleeding series and followed by a rest period before subsequent bleedings.

The bleeding procedure consisted of placing the rabbits in a restrainer and bleeding from the marginal vein of the ear by making a short, deep incision with a scalpel. (Figure 1). Prior to the incision the ear was cotton swabbed with xylene and 70% ethanol to dilate the blood vessels and cleanse the ear. Blood was collected in 50 cc. polyethylene centrifuge tubes. Blood volumes of 50 cc. per bleeding were average. The blood was allowed to stand at room temperature for one hour, and "ringed" with an applicator stick and then placed in the refrigerator from 6-12 hours. After centrifugation at 3,000 rpm for 50 minutes the antiserum was sterile filtered and placed in sterile 20 cc. serum vials. Antisera were then titrated, labeled and stored at -10° C.

Precipitin Tests

The ring test was used to determine the titer, which is the highest dilution of antigen in which a reaction can be observed. To obtain or determine the titer of an antiserum, serial dilutions of homologous antigen were prepared as follows:

Figure 1. Restrainer cage and equipment used in bleeding rabbits.



Figure 2. Photronreflectometer used to measure turbidity in precipitin tests.



Serial Dilutions Used to Titrate Antisera Against Weevil Extracts

<u>Tube</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>
Saline	4½	1	1	1	1	1	1	1	1
Undil. antigen (ml)	½	0	0	0	0	0	0	0	0
Transfer (ml)	1	1	1	1	1	1	1	1	1
Final Dilution	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560

After the dilutions were made, 0.2 cc. of each antigen dilution (tube) was pipetted into a small test tube and equivalent amounts of antiserum layered beneath with a Pasteur pipette. Ring tests were read against a black background with the light source in the foreground.

Photoelectric Measurements. The Libby photorelectrometer, a photoelectric instrument designed to measure turbidities of antigen-antibody reactions, was used to compare the weevil antigens (Libby, 1938) Figure 2. Serial dilutions of antigens were made in buffered saline, and 0.7 ml. of each antigen dilution was mixed with 0.3 ml. of undiluted antiserum. The resulting turbidity of each mixture was recorded in galvanometer units from the photorelectrometer and the sum of the turbidities (ST) for each series of mixtures was determined. For all tests the antigen had to be concentrated so the initial antigen dilution would approach zero. The antigens were concentrated by dialysis using polyvinylpyrrolidone (PVP). After the antigens had been concentrated to one-fourth the original volume, it was placed in a dialysis bag and suspended in physiological saline for three to four hours. During this time the saline was changed once.

Tests were made to determine if the concentration had any effect on the antigen that would cause inaccurate results when measuring the turbidities. By using concentrated antigen and making serial dilutions one-to-four (1:4)

one would expect to get the same turbidity values as were obtained using the undiluted antigen since the original volume was concentrated by one-fourth. The test showed this to be true. The following scheme was used for the turbidity measurements:

Tube	1	2	3	4	5	6	7	8	9	10	11	12
Saline (ml)	0	1	1	1	1	1	1	1	1	1	1	1
Concentrated Antigen	1	1	0	0	0	0	0	0	0	0	0	0
Transfer (ml)	0	1	1	1	1	1	1	1	1	1	1	1
Final Dilution	Conc.	1:2	1:4	1:8	1:16						1:1024

The homologous reaction was that which occurred between the antigen from one species and the antiserum produced when this antigen was injected into a rabbit. Heterologous reactions were those which occurred between the same antiserum and antigens from other related species of weevil.

Gel-Diffusion Analysis: Gel-diffusion tests were performed with weevil extract and concentrated antiserum. The antisera were concentrated by freezing the amount needed for each experiment in centrifuge tubes. After liquifying the antisera by centrifugation, the concentrated bottom layer was then used for testing. The reactions were performed in tubes (modified from Oudin, 1952) and in plates (Ouchterlony, 1949).

The method used for clarification of agar was modified from Feinberg (1956). The tubes were precoated with 1% agar and stored in a dessicator prior to use. Precoating was accomplished by filling the tubes with melted agar, and after 30 seconds pipetting the agar out and rolling the tubes on a hard surface to distribute the agar uniformly on the inner surface of the tubes. Precoating was necessary to keep the agar column from separating from the walls of the tubes during the reaction period.

In preparing the tube tests, 0.6% agar was melted in a water bath, filtered through glass wool in a Buchner funnel and placed into serum vials in a water bath (120° F.). Frozen anti-weevil serum was thawed and placed in the same water bath until both liquids were at the same temperature. Equal volumes of agar and antiserum were mixed together and the mixture pipetted into small tubes (3 mm. inside diameter and 75 mm. long) to within one inch of the top. This mixture was allowed to harden in the tubes at 5° C. for 10-15 minutes. The various concentrated weevil extracts were then layered over the hardened gel columns and the tubes were sealed with moulding clay. Reactions usually started within 48 hours and were completed in 2 weeks at room temperature. Readings were made approximately 150 hours after the tests were begun and daily thereafter until no further changes occurred. Tests were examined against a black background. Tests on each extract were recorded and performed in triplicate.

For agar-plate preparations, 1 per cent agar was melted, filtered through glass wool and 10 cc. poured into the bottom of each glass petri dish. After the bottom layer had hardened, circular well-moulds (penicylinders obtained from Fisher Scientific, 8 mm. inside diameter and 20 mm. high) were placed in the desired positions on top of the agar. The proper positioning of the penicylinders was accomplished with the aid of a well-pattern which was placed under the petri dish. A second layer of agar (10 cc.) was then poured around the well moulds and allowed to harden before removal of the moulds. The wells were then filled with antigens and antiserum and reactions were allowed to proceed at room temperature. The normal well pattern consisted of a center well filled with antiserum and a series of six evenly spaced wells in a circle around the center well. Different antigens were placed in these wells and the subsequent reactions of different antigens could be compared.

RESULTS

Ring Test

The endpoint or titer is represented by the highest antigen dilution giving a positive reaction. This procedure is used to detect specific antigens and gives little information regarding the content of the antiserum. This method does not suffer greatly from inhibition by antigen excess because diffusion at the interface provides a zone of nearly optimal proportions in which precipitation occurs.

The production of immune sera against all the weevils proved to be fairly easy, although the titers were not high. This however is not unusual with most insects. All titers were read and recorded after 60 minutes at room temperature. The following titers or endpoints obtained in the antigen dilutions were as follows:

Sitophilus oryzae -----1:640
Sitophilus zeamais -----1:1280
Sitophilus granarius -----1:1280

The purpose of the titer test in this study was only to secure comparable antisera and not to distinguish between species. This procedure, however would have practical value if homologous and heterologous reactions were performed. The ring test titer presents but a single point on a whole dilution series, and cannot be regarded as a true index of the similarity between antigens being compared. Therefore the entire reaction curve of antigens and antisera must be plotted if more critical analysis of animal relationships using serological methods are to be obtained.

Photronreflectometer Tests

Immune sera produced against species of the genus Sitophilus proved to be highly differentiating when used against members of that genus. A study of figures 3, 4, and 5 indicates that the rice weevils, Sitophilus oryzae and S. zeamais, are more closely related to each other than to the granary weevil, S. granarius. The antiserum against the granary weevil was very specific in that it resulted in heterologous curves that were less specific than with the other antisera. This indicates that the granary weevil is not as closely related to either of the two species of rice weevils as they were to each other. Reciprocal tests were performed using antiserum specific for the granary and for the rice weevil, S. zeamais, and in no instance was there any variation in the order of their relationships.

There is always the question as to the validity of experiments using extracts of whole insects as a basis for systematic classification. It is true that extracts of homologous structures in the insect body would theoretically present a more critical basis for comparison. However, the use of sera, or extracts of homologous structures, are not always practical when small organisms are being considered.

Antigens prepared as outlined in this study can be regarded as comparable representatives of these species of Sitophilus because: (1) the extraction procedure selects out only the more soluble antigens, (2) the rabbit responds only to the better antigens, (3) antibodies react specifically only with corresponding antigens, and (4) reciprocal tests provide ample evidence for the validity of the relationships, (Boyden and DeFalco, 1943).

Leone (1947a) stated that the objections to the worth of reciprocal serological tests whose degree of correspondence varies may be due to many

Figure 3. A photronreflectometer curve series titrated against an anti-Sitophilus oryzae serum. Percentage values indicate the relative degrees of correspondence of the homologous antigen Sitophilus oryzae, demonstrated by the two heterologous antigens.

Figure 3

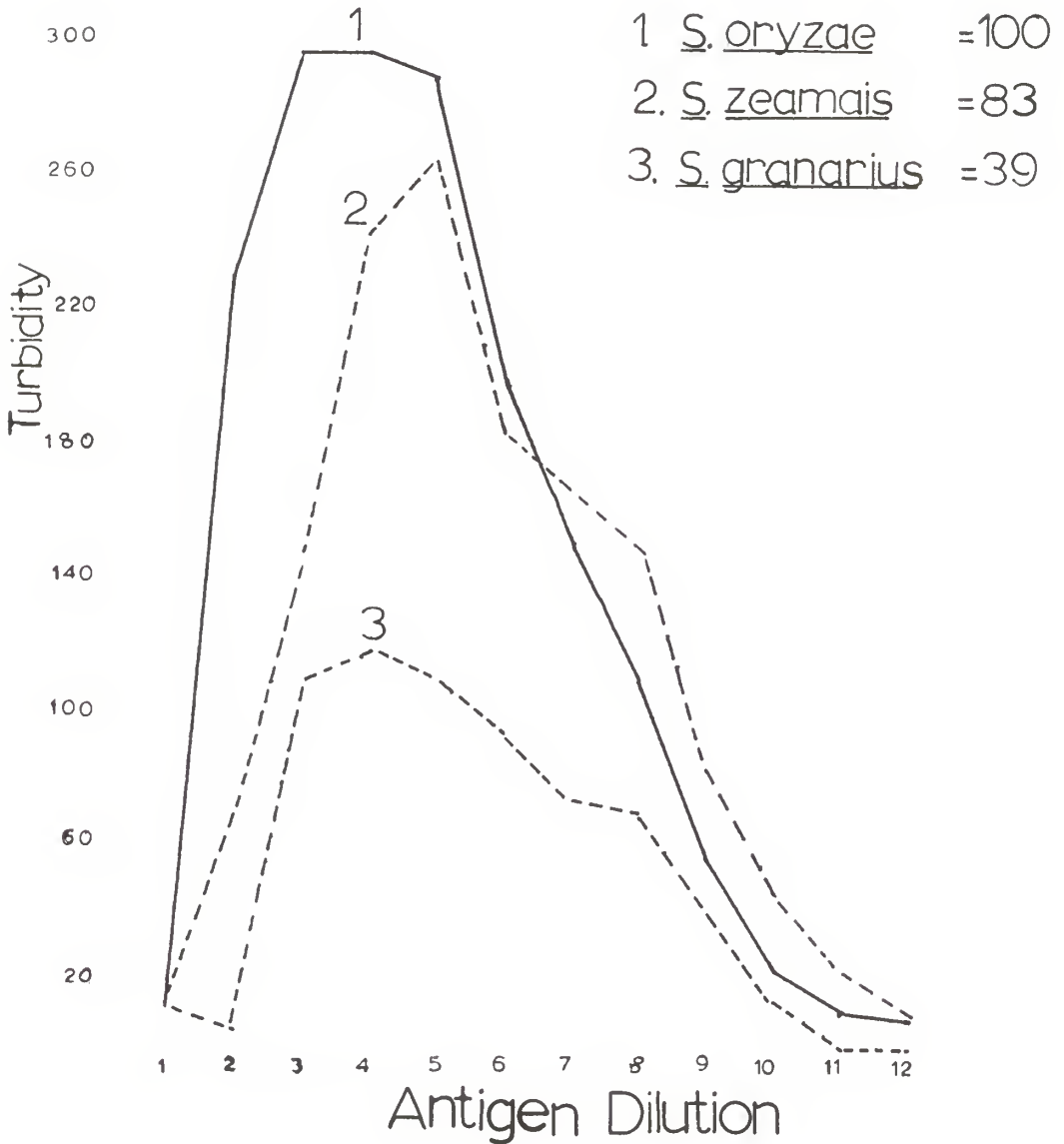
Anti-S. oryzae

Figure 4. A photronreflectometer curve series titrated against an anti-Sitophilus zeamais serum. Percentage values indicate the relative degrees of correspondence of the homologous antigen S. zeamais, demonstrated by the two heterologous antigens.

Figure 4

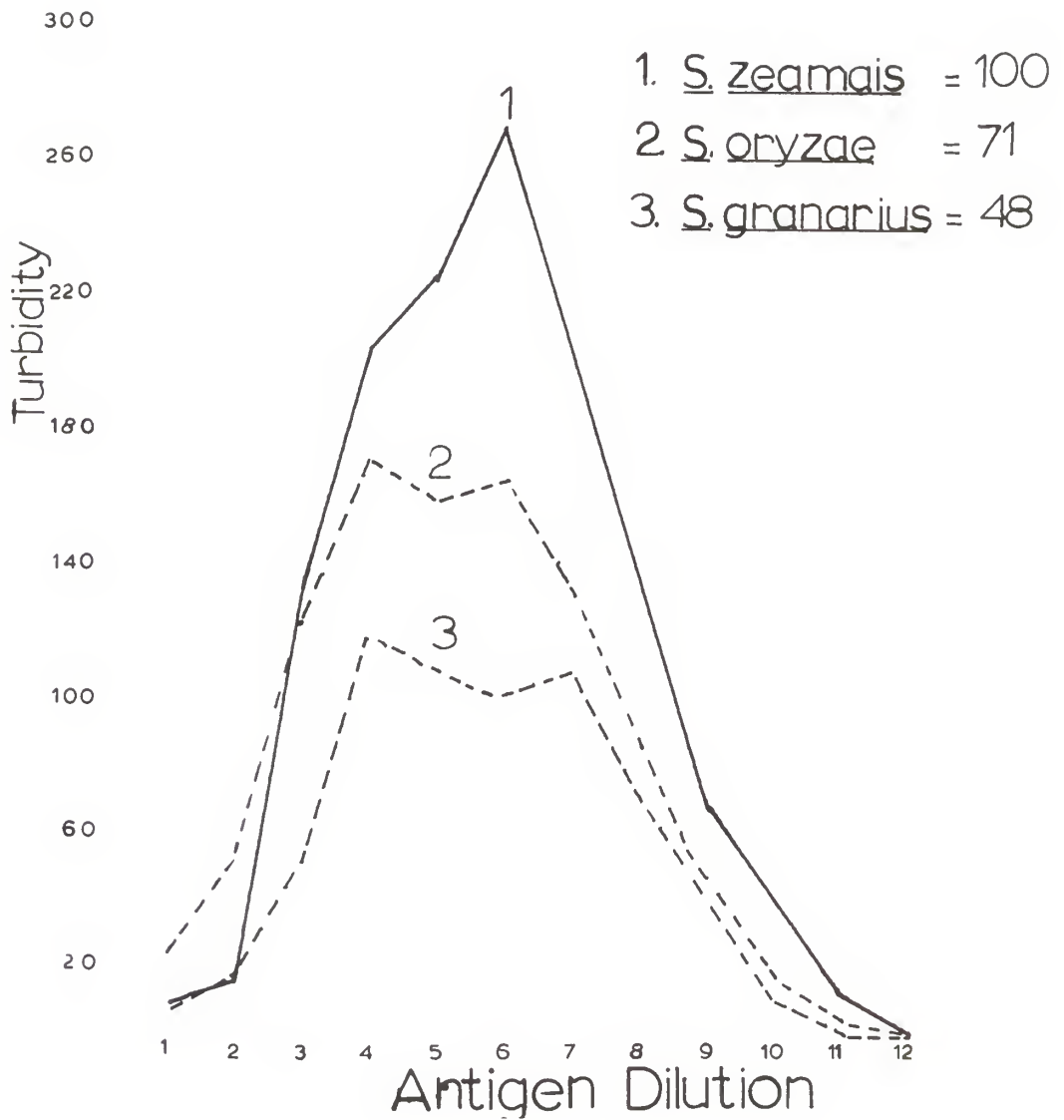
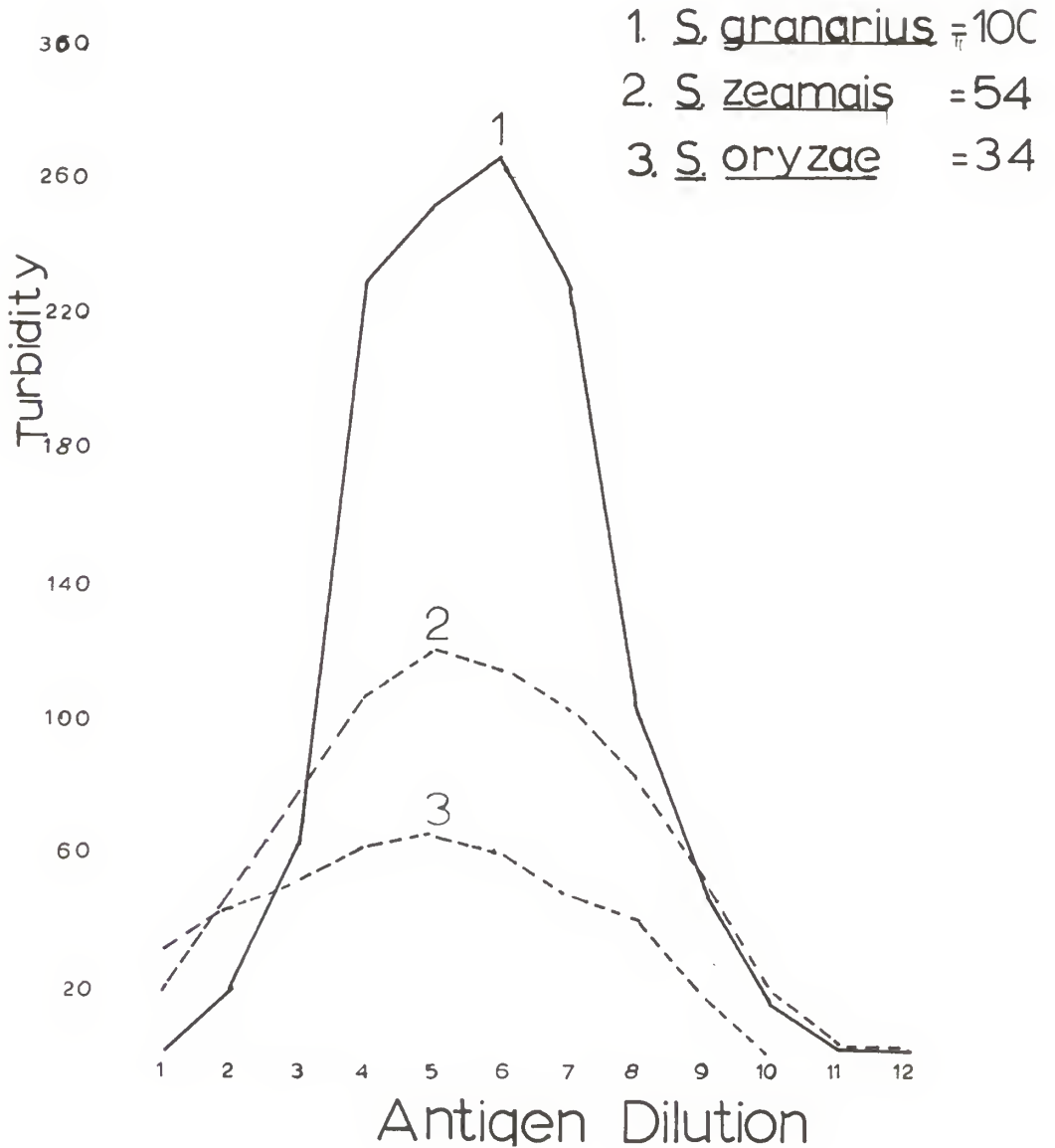
Anti-S. zeamais

Figure 5. A photorefractometer curve series titrated against an anti-Sitophilus granarius serum. Percentage values indicate the relative degrees of correspondence of the homologous antigen S. granarius, demonstrated by the two heterologous antigens.

Figure 5

Anti- S. granarius

factors. He also stated that the greatest variable in any serological problem is the animal that is used for the antibody production. The different percentage relationships between organisms in reciprocal tests are the result primarily of the variability of rabbit response, which, in turn, is a function of the animals physiological condition. Boyden and DeFalco (1943) have presented information describing the nature of antisera behavior in precipitin systems. The lack of exact correspondence of the reciprocal tests is not a valid reason for discrediting the use of extracts of whole organisms in establishing relationships, or for not employing serological methods to classify insects (Leone, 1947a).

Gel-Diffusion Analysis

Since antibodies are not homogenous and since there may be many antigens present in even the purest preparations, there are several possible antigen-antibody reactions in a single mixture. The gel-diffusion technique permits the examination of such multiple systems since this technique facilitates the separation of these reactions and since the individual reactants in a system react independently of each other.

On this analysis the gel, which is clarified agar, serves as the matrix for combining diffusion with precipitation. The reactants simply diffuse through the gel towards each other and precipitation results when the equivalence points have been reached. A single antigen will give rise to a single line of precipitation in the presence of its homologous antibody. When two antigens are present in a system, each behaves independently of the other. Thus, if several bands of precipitation are evident, there are at least that many antigen-antibody combinations present.

The coalescence of zones provides a means for identification of antigens. If two antigens diffusing from parallel sources form zones of precipitate which coalesce completely, they may be considered identical; if the zones cross, the two antigens are different. Naturally as long as the zones of precipitation fail to reach one another, we can conclude nothing. This may occur in cases in which antibody concentration is very low and the concentrations of antigens are very high, so that the two zones of precipitation are short, move quickly away from the sources of antigen and reach the antibody source before their extremities have united.

Plate tests proved to be less sensitive than the tube tests; the number of bands were less in plate tests than in tube tests (Figures 6A, B, C).

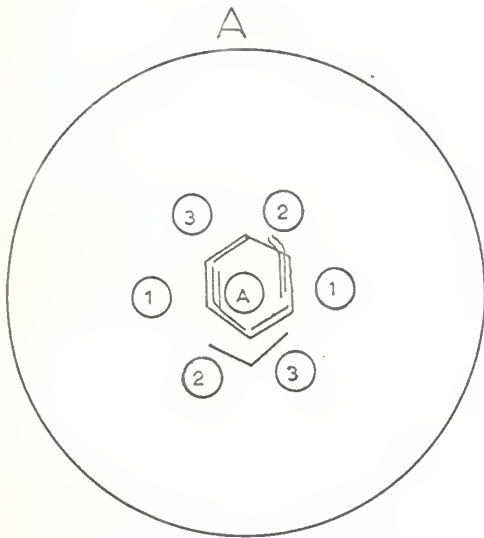
Examination of all plates revealed that there was at least one antigen common for all three of the weevils tested. With certainty the plate tests showed antigens specific for one species and not for the others.

Gel-Diffusion Tube Test. This technique is based upon the fact that soluble antigen, when layered over semisolid mixture of antiserum and agar in a narrow tube, will diffuse through the semisolid mixture and form bands of precipitate that represent antigen-antibody reactions.

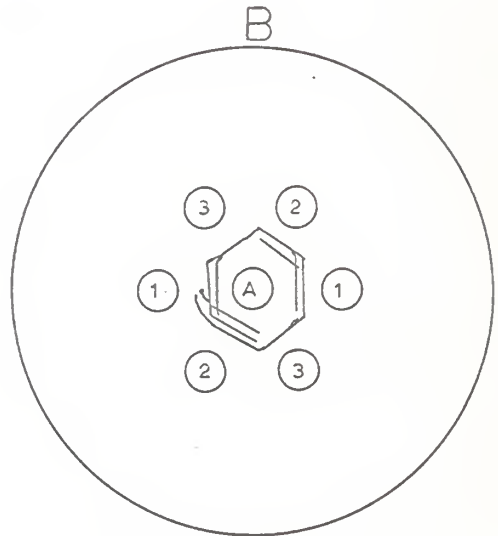
Antiserum-agar tube tests were performed with saline extracts of adult weevils. The interfacial titer was the same as was indicated previously. The tubes were examined 150 hours after the tests were set up and at intervals until the reactions were complete.

The results of the interfacial titers and antiserum-agar tests with the three weevils are summarized in Table 1. The precipitin test comparisons of antigen-antibody reactions show that there is a degree of specificity which can be interpreted as indicating biochemical differences.

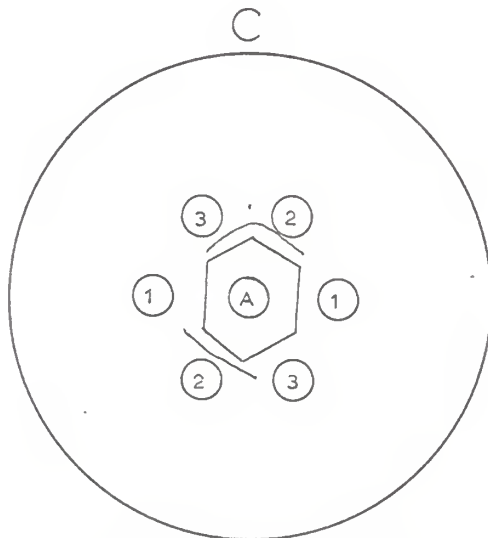
Figure 6. Agar-plate Tests with Weevil Extracts



A- anti-S. oryzae serum
 1- S. zeamais antigen
 2- S. oryzae antigen
 3- S. granarius antigen



A- anti-S. zeamais serum
 1- S. oryzae antigen
 2- S. zeamais antigen
 3- S. granarius antigen



A- anti-S. granarius serum
 1- S. zeamais antigen
 2- S. granarius antigen
 3- S. oryzae antigen

Table 1. Interfacial titer tests and numbers of precipitate bands appearing in antiserum-agar reactions with adult Sitophilus spp. weevil extracts.

Weevils	Titer	No. of precipitate bands
<u>S. oryzae</u>	1:640	19
<u>S. zeamais</u>	1:1280	18
<u>S. granarius</u>	1:1280	16

Qualitative comparisons of the antigenic content of different solutions can be made by counting the number of bands appearing when each solution is added to a tube of the same type of antiserum-agar. However, this procedure does not eliminate the possibility that two bands may advance through the antiserum-agar at identical rates and thus appear as a single band of precipitate, or that a band of low density may advance at a slower rate than a band of high density and therefore be obscured (Oudin, 1948). Furthermore, if each of two solutions contains an antigen which the other does not contain, these solutions could cause the appearance of the same number of bands of precipitate despite their qualitative difference. Therefore, adequate qualitative comparisons of different antigen solutions have to include absorption tests which permit one to locate and identify the corresponding bands of precipitate in different tubes (Telfer and Williams, 1952).

DISCUSSION

The results presented are only a beginning in the study of the problem of the quantitative serology of stored-grain weevils. The insect species used in the tests were chosen because of their availability to the writer and because weevils of the rice-weevil complex are not identifiable by external characters. It was desired to know the type of serological results that could be obtained using extracts of whole insects as antigens characteristic for the species tested.

The results do indicate that quantitative serologic analysis can be used to separate species of the genus Sitophilus.

The relationships shown by serological techniques are in general agreement with those indicated by comparative morphology. The two rice weevils were the most antigenically similar and could be differentiated from the granary weevil.

It should be pointed out that the percentage values found using the photoneutron reflectometer are not absolute but only relative. Leone (1947) suggests that more data must be accumulated on actual systematic tests before serological limits in terms of percentages can be summarized or defined as representing species of insects.

Boyden (1963) offers the following three potential contributions of precipitin testing to systematics:

1. Give an objective relative placement series based on the amounts of biochemical similarity in the surface of protein antigens, with the homologous antigens as standard of reference.
2. Place species and larger groups of existing organisms with reference to each other in a multidimensional plot.
3. Provide data, which, together with all other available data in regard to the nature of organisms, can be used as the basis for a truly

natural system for the classification of existing organisms.

In evaluating the value of the different tests used in this study, the turbidimetric measurements would have to be considered the most useful because the tests cover the whole antigen reaction ranges of the antiserum.

It should be noted that this study in no way represents the final answer, but furnishes information on the quantitative relationships of species of Sitophilus. This information may be helpful to the systematist. In general, the relative intensities of precipitin reactions parallel the systematic position of species compared where that position has been established by other means.

The precipitin test is a firmly established procedure and there can be no question as to the validity of the principle of quantitative specificity.

There are many problems relating to the systematics of various of the stored-grain insect groups which may be clarified by application of the precipitin technique. Certain of the flour beetles and populations of the rice-weevil complex are but two of the possibilities. It would seem probable that serological studies will play an increasingly important role in stored-grain insects research.

SUMMARY

1. Three species of adult grain-infesting weevils of the genus Sitophilus, were subjected to precipitin tests to determine if they could be differentiated using serological techniques.
2. The two rice weevils, Sitophilus oryzae and S. zeamais, were the most antigenically similar and could be easily differentiated from the closely related granary weevil, S. granarius. The two rice weevils also could be separated.
3. The relationship shown by serological techniques are in general agreement with those indicated by comparative morphology.
4. Serological techniques should prove of value in studying geographical populations of species-complexes in the genus Sitophilus.

ACKNOWLEDGMENTS

The writer expresses grateful acknowledgment for the assistance and guidance of Dr. A. E. R. Downe and Professor Donald A. Wilbur in the conduct and execution of this research. Appreciation is also expressed for the special assistance of Dr. Charles A. Leone, Dr. John B. Edman and Dr. Robert B. Mills. Thanks are also due Dr. Yesoda Raj, Miss Sue Shelton and Mr. David Lodge for valuable technical assistance.

Support was generously given by the Department of Entomology, Kansas Agricultural Experiment Station, Manhattan, Kansas and by Grant EF-114 U. S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, Division of Environmental Engineering and Food Protection.

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SEROLOGICAL COMPARISON OF THREE SPECIES
OF GRAIN INFESTING WEEVILS
(CURCULIONIDAE: SITOPHILUS)

by

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B.S., Kansas State University, 1963

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

Kansas State University
Manhattan, Kansas

1965

ABSTRACT

Studies on differentiation of three species of grain-infesting weevils were performed, using serological techniques. Adult weevils were reared in the laboratory and subjected to precipitin tests with homologous and heterologous antisera. In this manner differentiation or divergence among the three species was determined. The approach was to prepare specific antisera against each species and determine if they were physiologically separate. Antisera were produced in rabbits against the specific antigen extracts.

Turbidity measurements using the Libby photorefractometer indicated that the weevils were distinguishable by precipitin reactions. Essentially this procedure involved the measurement of the turbidity developed in fluid precipitin tests in which each antiserum in constant amounts was tested against a doubling dilution series of antigens. Sitophilus oryzae and S. zeamais, the two rice weevils, were more antigenically similar than the closely related granary weevil, S. granarius.

Non-identical antigens were compared by observing the patterns of intersection of precipitates with a common antiserum in gel-diffusion plate tests. Patterns of fusions, intersection, and partial intersection were interpreted in terms of antigenic similarity of the antigens. These tests also supported the serological correspondence found, using turbidity measurements.

Antiserum-agar tube tests carried out in Oudin tubes which measured the minimum number of precipitate bands also supported the turbidity measurements. Bands of precipitate formed represented a separate antigen-antibody reaction which is indicative of the various protein systems present. A comparison of the number and similarity of the bands indicated that possibly different proteins were present for each of the three species.