SALMONELLA MONTEVIDEO: ITS EFFECTS ON, AND TRANSMISSION BY, THE LESSER RICE WEEVIL (*SITOPHILUS ORYZAE* (L.)) IN WHEAT

by

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INTRODUCTION

As early as 1602, Aldrovandi (Greenberg and Bornstein, 1964) recorded that Greeks and Romans were aware of the possible spread of dysentery by the ingestion of flies accidentally falling into food. Protecting food from harmful microorganisms is important and the potential role of stored-grain insects in transmission of Salmonella is presented.

The Food and Drug Administration (Lennington, 1967) reported that flies, roaches, ticks, fleas, and other insects are active in the chain of Salmonella infection. They did not test grain for Salmonella unless it was seized for some other reason, but, if Salmonella organisms were found, the shipment would not be used even for livestock feed.

Salmonella

The first isolation of a member of this genus was done by Salmon and Smith in 1885 from swine (Galton, Steele, Newell, 1964). Salmonella montevideo, the serotype used in these experiments, was originally isolated from human sources in Montevideo, Uruguay. It was also isolated from an ape, hogs, and chickens (Bergey, 1948). S. montevideo was the second most common Salmonella serotype isolated from eggs

and egg products in 1966, but it has also been found in various type of animal feeds and vegetables. It is usually found more in non-human isolations but it has been the cause of some epidemics of gastro-enteritis in the United States and other parts of the world (Buchner, 1967).

Lesser Rice Weevil (Curculionidae, Coleoptera)

The lesser rice weevil, Sitophilus (Calandra) oryzae (L.) was described in 1763, after being obtained from rice in Surinam (Cotton, 1963). The weevil is one of the most serious and common of the stored-grain pests. It is approximately 3 mm in length with the head prolonged into a long slender snout with the mandibles at the end.

Objectives

1. Determine the effects of Salmonella montevideo on the longevity of adult rice weevils.

2. Determine the effect of S. montevideo on the number of adult progeny produced by weevils reared in S. montevideo-contaminated wheat.

3. Develop a technique to surface-sterilize adult rice weevils and other stored-grain insects so that S. montevideo present internally could be isolated.

4. Determine the ability of adult rice weevils to transmit S. montevideo from one sample of wheat to another.

5. Determine the survival of S. montevideo in wheat over a 21-day period and how this affects the ability of rice weevils to pick up S. montevideo.

REVIEW OF LITERATURE

Salmonellae and stored-grain insects have each been known to be a problem in food sanitation for many decades, but the relationships between them has not been studied. Therefore, the literature dealing with transmission of Salmonellae by insects does not include stored-grain insects and their ability to transmit these organisms.

Transmission of Salmonellae by Insects

Felt, in 1908, was one of the first to accumulate experimental evidence linking Salmonellae with flies. It was not until 1942 that investigations in Argentina by Hormaeche, Peluffo, and Aleppo (Peluffo, 1964) began to establish the rate of occurrence of enteric bacteria in flies. They found that 85 of 362 pools of flies yielded over 15 types of Salmonellae during the summer months and that these types were similar to those isolated from children's diarrhea in the same period of time.

Beck (1943) reported that Salmonella typhimurium was disseminated by cockroaches of the genus Periplaneta americana but not by the genus Blatella germanica. Periplaneta harbored the Salmonella in the intestine as well as on the appendages.

In Australia, Mackerras and Mackerras (1949) reported that several roaches had Salmonellae in a hospital where there was an epidemic of infantile gastro-enteritis. They concluded that lack of high incidence of infection in the roaches (4 of 106) could be regarded only as a reflection of the opportunities the roaches had to acquire and disseminate infections in the wards. In San Antonio, Texas, Bitter and Williams (1949) reported that four cockroaches taken from sewer manholes were infected with Salmonellae.

Olson and Reuger (1950) conducted experiments on the transmission of Salmonellae by cockroaches in order to determine their potential as vectors. Survival of Salmonella in the insects was determined by examination of the feces. It was found that Salmonella could survive in the American roach 10 days, the German roach 12 days, and the Oriental roach 20 days. A post mortem examination of Oriental roaches showed them positive 42 days after an infective feeding. Fecal pellets remained infective for 199 days at room temperature.

Ostrolenk and Welch (1942) successfully transferred S. enteritidis from infected house flies to mice and from the mice back to flies. The infected flies were found to infect other flies, food, water and miscellaneous surfaces with which they came in contact. The Salmonella was able to survive in the digestive tract throughout the adult life of the fly.

Watt and Lindsay (1948) conducted insecticidal control studies in a community and found that the control measures significantly reduced the incidence of infection, but that reduction was greater with Shigella infections than with Salmonella.

Greenberg et al. (1963, 1964) revealed that 12 types of Salmonella were recovered from flies around slaughterhouses in Mexico and that flies could transmit Salmonellae for at least 3 miles. They also found that S. typhimurium was unable to survive metamorphosis in blowfly larvae, and that S. typhi was not able to survive in the house fly while S. enteritidis and S. paratyphi could survive.

Stored-grain Insects as Hosts for Micro-organisms

Van Wyk, Hodson, and Christensen (1959) isolated large numbers of bacteria from larvae and adults of the confused

flour beetle, Tribolium confusum (Duv.). More bacteria/gm were isolated from insects than in the food from which the insects were taken. When T. confusum and the rice weevil were reared together in wheat of 16-17% moisture content, the populations of bacteria in the grain increased greatly.

Misra, Christensen, and Hodson (1961) consistently isolated storage fungi from surface-disinfected Angoumois grain moth (Sitotroga cerealella (Oliv.)) larvae, pupae, and adults and from the alimentary tract of the larvae. Kantack (1963) noted similar results with Trogoderma glabrum (Herbst) larvae. Sirkorowsky (1964) showed the red flour beetle, Tribolium castaneum (Herbst) could carry two species of fungi internally as well as externally. The flat grain beetle, Cryptolestes pusillus (Schonh.), and the saw-toothed grain beetle, Oryzaephilus surinamensis (L.) were also found to be hosts of at least one species of storage fungi.

MATERIALS AND METHODS

General Methods

Lesser rice weevils, Sitophilus oryzae (L.), were reared in 1-quart jars containing Ponca hard red winter wheat (1966 crop) with approximately 13.6% moisture content. The jars

were kept in a chamber (83 cm x 95 cm x 80 cm) at 27°C (± 1°) and 70-75% relative humidity (Plate I, Fig. 1). The relative humidity was controlled by using a pan of water partially covered with a cardboard strip and was measured with a Bendix aspirated psychrometer. Moisture content of the wheat was determined with a Motomco electronic moisture tester calibrated by the air oven method.

Two-hundred gm quantities of wheat in quart jars were inoculated with Salmonella montevideo for use in these experimental studies. The Salmonella organisms were grown on proteose peptone agar slants and were harvested for use after growth for 18 hr at 37°C. The organisms were scraped from the slants and suspended in sterile 0.1% tryptone (Difco). The suspension approximated 200 times the Nephelometer #1 turbidity. Each jar containing 200 gm of wheat was inoculated by adding 5.0 ml of Salmonella montevideo suspension. To distribute the organisms over all kernels the jars were rolled on a U. S. Stoneware Roller until the wheat ceased to adhere to the sides of the jar. The jars of wheat were then placed in the rearing chamber for at least 24 hr before insects were added.

Seven gm of S. montevideo-contaminated wheat were transferred to each covered plastic box (5 cm x 5 cm x 2 cm) (Plate I, Fig. 2). An equal number of boxes with

EXPLANATION OF PLATE I

Fig. 1. Chamber which contained wheat samples and insects for experiments.

Fig. 2. Plastic boxes which contained the 7-gm wheat samples and 15 weevils.

PLATE I

Fig. 1

Fig. 2

uncontaminated (clean) wheat served as controls. The wheat samples were weighed on an Ohaus triple-beam balance.

Longevity and Progeny of Weevils in Contaminated Wheat

To determine if Salmonella montevideo in the culture wheat would affect the longevity of adult rice weevils and the number of adult progeny produced, 10 female and 5 male adult weevils (0-9 days after emergence) were put in each of 3 plastic boxes of S. montevideo-contaminated wheat and 3 boxes of clean wheat. Shuco-Vac vacuum tweezers were used to pick up and transfer the weevils (Plate II, Fig. 1). They were left in these boxes for one week after which the weevils from each box were transferred every 7 days to another plastic box containing the same type of wheat. Dead weevils were removed at each transfer and the date recorded.

Approximately one month after beginning the test, adult progeny began to emerge from the kernels in the first plastic boxes infested. The weevils were removed every 24-48 hr from each box for 2 weeks after the first emergence. Weevil removal was discontinued for each box at the end of the 2-week period since most of the progeny had already emerged. This procedure was followed for each box while periodically checking the weevils for S. montevideo externally and in the digestive tract.

EXPLANATION OF PLATE II

Fig. 1. Shuco-Vac vacuum tweezers for picking up and transferring rice weevils.

Fig. 2. Digestive tract of lesser rice weevil. Left to right are part of the exoskeleton of thorax, digestive tract, and part of the exoskeleton around the anus.

PLATE II

Fig. 1

Fig. 2

The boxes of contaminated wheat were also tested for S. montevideo at the end of the 2-week period. The 7-gm wheat samples were tested by pouring the sample into 30 cc of brilliant green tetrathionate broth and allowing it to incubate for 24 hr. Some of the incubated broth was streaked on a brilliant green agar plate and incubated for 24 hr.

Surface-sterilization and Dissection

Five hundred 7-week-old adult weevils were placed in a gallon jar containing 500 gm of wheat contaminated with S. montevideo. Near the end of the test, deterioration of the grain in the jar was almost complete so another jar of contaminated wheat was infested with 500 weevils to provide the insects needed to finish the test. Over an 8-month period, techniques were developed to surface-sterilize the rice weevils and to recover S. montevideo from the digestive tracts (Plate II, Fig. 2). It was necessary to revise the technique several times; the following was most effective:

1. Instruments and glassware were sterilized in autoclave at 121°C and 18 lb pressure for 20 min.
2. All work surfaces were washed with 1% solution of mercuric chloride, including the microscope.

3. The number of needed weevils was removed with forceps from the culture jar and placed in a sterile petri dish (100 mm x 15 mm). Forceps were flamed 3 times over an alcohol burner before and after handling insects. These weevils were killed by placing them in a freezer for at least 1 hr.

4. After 1 hr, 10 of the weevils were placed in a vial containing proteose peptone broth. This vial was labeled "control."

5. The mouthparts and anus of each of the remaining weevils were covered with Vaseline petroleum jelly. This was done along with freezing the insects to prevent S. montevideo, which might be inside the insect, from coming out during or after surface-sterilization.

6. The weevils were placed in a petri dish containing 70% ethyl alcohol for at least 5 min.

7. The weevils were removed from the alcohol and placed in a petri dish and covered with filter paper. The filter paper was covered with 1% solution of mercuric chloride with Tergitol for at least 15 min.

8. The mercuric chloride solution containing the insects was poured through a funnel containing filter paper. Sterile water was poured over the weevils twice to wash off the mercuric chloride.

9. Weevils were removed from the filter paper gently to avoid breaking the exoskeleton, then placed in sterile water and carefully agitated for 5 min. The water and insects were poured into another funnel with filter paper.

10. Weevils from funnel were removed and put on dry filter paper. Ten weevils were placed in a proteose peptone broth vial to test for effectiveness of surface-sterilization. The remainder of the weevils were placed in a petri dish containing

Tissuemat (paraffin-like wax with a melting point of 56.5°C). The Tissuemat was melted and the dorsum of each weevil was embedded in the melted wax.

11. Weevils were covered with sterile water for easier dissections.

12. Dissecting needles and forceps were flamed 3 times before and after dissections.

13. The digestive tracts were severed near the anus and close to the head. After removing at least two thirds of the tract of each weevil, 10 of the tracts were placed in each proteose peptone broth vial. Usually the tracts were removed in pieces which exposed more of the contents in the tract to the broth. The vials were placed in a 37°C chamber for 24-72 hr.

14. The contents of the vials were then streaked on a brilliant green agar plate and incubated for 24 hr. The presence or absence of S. montevideo was observed. If S. montevideo was absent the vials were incubated an additional 48 hr and new plates were streaked.

15. The petri dish containing the remains of the weevils was autoclaved after the used Tissuemat was put in a jar of mercuric chloride for 1 hr and then discarded.

Transmission of S. montevideo by the Lesser Rice Weevil

Tests were conducted to determine whether rice weevils could transfer Salmonella montevideo from contaminated to clean wheat and, if so, what length of exposure to the

S. montevideo-contaminated wheat was necessary to enable transmission to the clean wheat. Fifteen adult weevils (0-9 days after emergence) were placed on 7 gm of contaminated wheat in each of 27 plastic boxes (5 cm x 5 cm x 2 cm).

Weevils were retained in 9 boxes for 1 week, in 9 other boxes for 2 weeks, and 9 more for 3 weeks. These will be referred to as the first, second and third sets, respectively. The weevils were removed from 3 of the boxes of each set after the designated time. These weevils were checked for presence of S. montevideo externally and in the digestive tract.

The remainder of the weevils from each set (6 boxes/set) were then taken from the boxes of contaminated wheat and placed in boxes of clean wheat. One week later, weevils from 3 boxes of each set were removed and checked for S. montevideo. The wheat from which the weevils were removed was also tested for S. montevideo. The weevils in each of the 3 remaining boxes in each set were removed and transferred to another box of clean wheat every 7 days. Each time the weevils were transferred, the wheat from which they were taken was tested for S. montevideo. This procedure was continued until the wheat indicated absence of S. montevideo. This gave an indication of how long the adult weevils could continue to transmit S. montevideo to clean wheat.

Persistence of S. montevideo in Wheat Infested with Lesser Rice Weevils

An experiment was conducted on the persistence of S. montevideo on wheat over a 21-day period and how this affects the ability of the rice weevil to pick up the bacteria. Two 1-gallon jars with 500 gm of wheat were contaminated with a heavy suspension of 18-hour-old S. montevideo cells and 500 newly-emerged adult weevils (0-7 days) were added 5 hr later. Ten gm of wheat and 40 weevils were removed 24 hr after the wheat was contaminated and checked for S. montevideo. The number of bacteria cells in each jar was combined and an average of the 2 jars was recorded. This procedure was repeated every 3rd day until the 21st day.

Procedure for checking the wheat differed in this test since the actual number of S. montevideo recovered was important. The 10-gm sample was poured into a dilution bottle containing 90 ml of sterile water and shaken for 2 min. Subsequent dilutions were made and 0.1 ml of each dilution was spread on brilliant green agar plates and incubated for 24 hr.

RESULTS AND DISCUSSION

Longevity and Progeny of Weevils in Contaminated Wheat

Longevity of adult rice weevils in S. montevideo-contaminated and uncontaminated (clean) wheat was checked weekly when they were transferred to other boxes of wheat. When a weevil was found dead at the end of the 1-week period, the date recorded for the death was the date the weevil was placed in the box rather than the end of the 1-week period. Figure 1 shows a comparison of the survival in contaminated and uncontaminated wheat.

Average survival of adults was greater in contaminated wheat than in clean wheat. Major differences appeared after 7 weeks. In contaminated wheat there was an average of 14.7 weevils/box alive at 7 weeks; at 8 weeks, 13.7/box; at 11 weeks, 11.0, and at 14 weeks, 6.5. The weevils in the clean wheat starting with the 7th week had an average of 14.0 weevils alive/box. The 8th week average was 10.0; the 11th week was 7.3, while at the 14th week only 3.3 weevils were alive. Starting with the 15th week the mortality rates were nearly the same for both groups. These results indicate the weevils in the contaminated wheat had a slower death rate than did the control insects in the clean wheat; therefore,

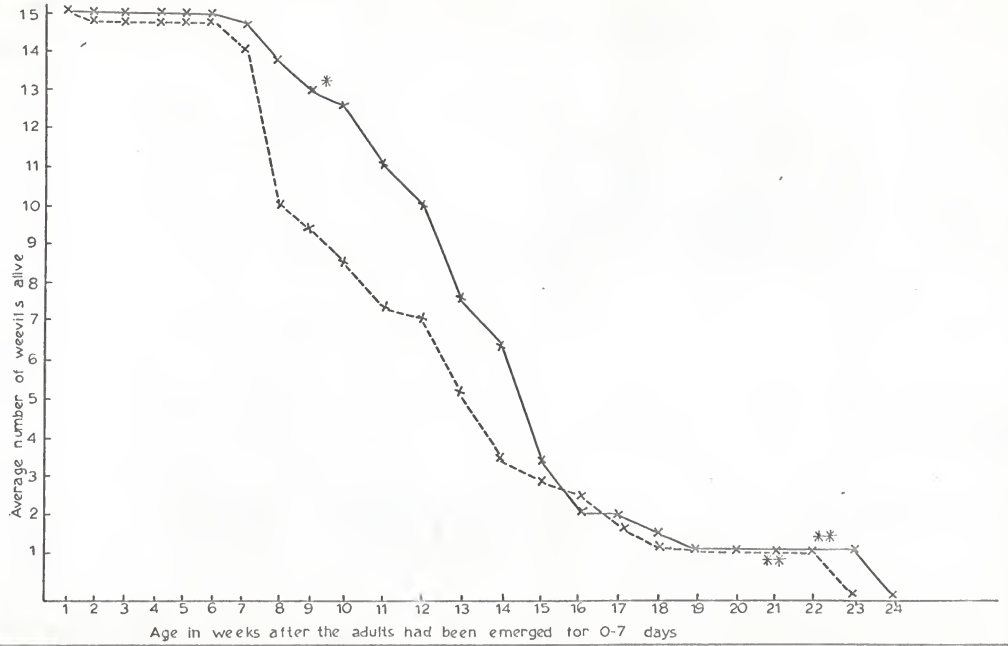
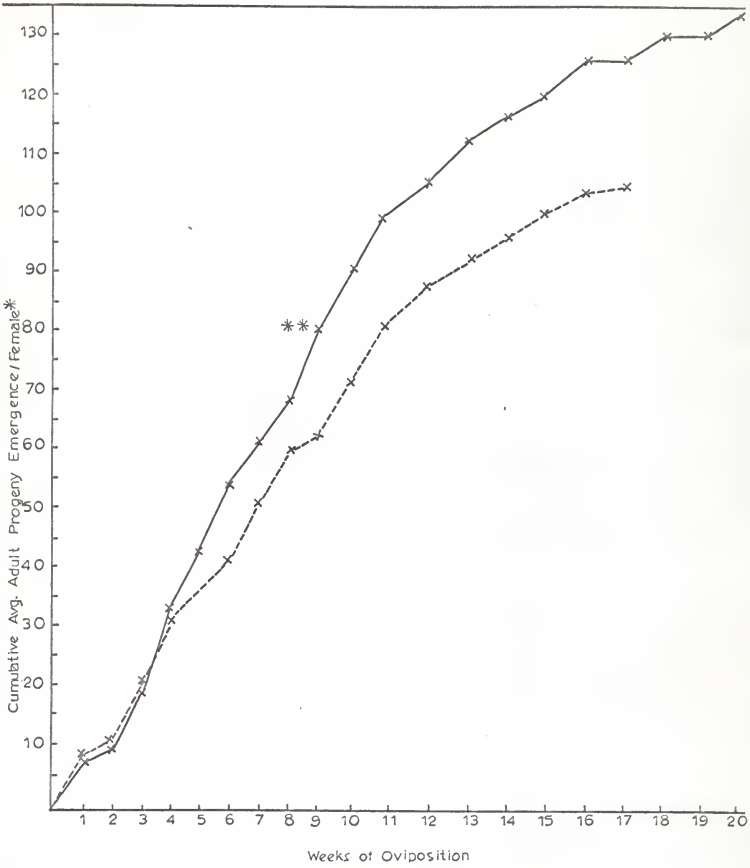


Fig. 1. Longevity of adult weevils in Salmonella montevideo-contaminated and clean wheat (7 gm/box). (Avg. of 3 replicates)

the presence of S. montevideo did not shorten the life of the adult weevils. However, the 5.0 ml of solution which was added to the wheat to inoculate it with Salmonella montevideo may have provided enough moisture to be the cause for increased longevity of the weevils.

S. montevideo did not adversely affect the number of adult progeny produced by the rice weevils in the longevity test (Fig. 2). The number of progeny removed from each box of wheat during the 2-week emergence period was divided by the number of females present at the end of the oviposition period. This number was then averaged with the other replicates to determine the average number of adult progeny/female which emerged. The total number of progeny/female which emerged was higher in wheat contaminated with S. montevideo (133.7) than in the control (103.5). The moisture content of the wheat which was raised initially at inoculation may have been the cause for more progeny in the contaminated wheat. Some of these progeny, which were removed at least every 48 hours, tested negative for Salmonella (11 of 17) even though they were reared in contaminated wheat.

Data were recorded on a comparison between the weevils in the contaminated and clean wheat on the lengths of time



* Adult progeny were collected from each box for 2 weeks after first emergence
** Remainder of line is average of 2 replicates
×——× Weevils in contaminated wheat ×------× Weevils in clean wheat

Fig. 2. Cumulative record of adult progeny/female (avg. of 3 replicates) during successive 1-week oviposition periods in boxes of contaminated and clean wheat. (7 gm/box; weevils transferred to new box after 1-week oviposition period).

from transfer of weevils to each box of wheat to the first day of adult emergence in that box (Fig. 3). In most instances, this period of time was shorter and more consistent for the weevils in the contaminated wheat. Here again, the increased moisture content in the contaminated wheat may have been the cause for the shorter time. Both groups, however, indicated a longer period to the first emergence from the first two 1-week oviposition periods (39 days), then a shorter time from the 4th through the 10th oviposition periods (not above 32), and then an increase from the remaining 1-week oviposition periods.

Surface-sterilization and Dissection

During the 8-month period of developing an efficient surface-sterilization technique, several changes were made. The changes listed under methods in Table 1 refer to the adding of a step while retaining the methods listed above them unless otherwise stated. Every group of 10 weevils which was taken directly from contaminated wheat and put in broth for each of the tests was positive for S. montevideo. The number of these positive groups used is given in the second column of Table 1. Brilliant green tetrathionate broth (Salmonella-selective media) was replaced by proteose broth in order to enhance the growth of the bacteria.

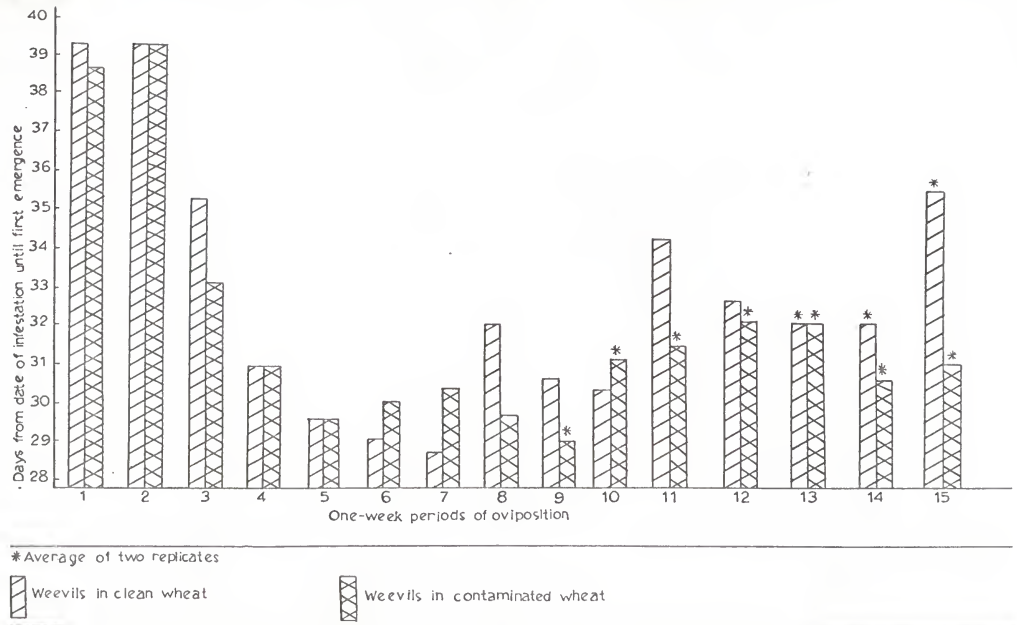


Fig. 3. Length of time from infestation with adult weevils to first emergence of progeny in contaminated and clean wheat (7 gm/box). Parent weevils transferred to boxes of fresh media after each 7-day oviposition period. Average of three replicates.

Table 1. Presence of Salmonella externally (E) and in the digestive tracts (DT) of the weevils after surface-sterilization. Each number represents the number of samples. Each sample consisted of 10 insects; in the last column each sample consisted of digestive tracts dissected from 10 weevils.

Method of surface sterilization and revisions	No. of positive samples tested	Present E (technique ineffective)	Not present E No. of DT samples positive/No. of samples of dissected weevils*
In $HgCl_2$ solution for 15 min	5	1	1/4
Mouthparts, anus covered with Vaseline petroleum jelly before placed in $HgCl_2$ solution	18	1	2/12
$HgCl_2$ solution was changed to include 0.1% Tergitol (a wetting agent)	2	0	0/2
Weevils placed in proteose peptone broth instead of brilliant green tetrathionate broth	7	1	1/6
Weevils taken from a second jar of freshly contaminated wheat (technique same as above)	6	3	2/3
Weevils from second jar placed in 70% ethyl alcohol for 5 min before placed in $HgCl_2$ solution	9	1	6/6

* In two instances not all of the samples of weevils were dissected after surface-sterilization.

Transmission of S. montevideo by the Lesser Rice Weevil

The weevils in the contaminated wheat for 7, 14, and 21 days tested positive for S. montevideo both externally (weevils not surface-sterilized) and in the digestive tract (Table 2). Each of the groups was positive externally even after being in clean wheat for 7 days, but only weevils in the 14 and 21-day groups tested positive externally and in the digestive tract after 5 weekly transfers to clean wheat. This seemed to indicate a direct relationship between the ability of the weevil to retain S. montevideo and the length of time the weevils were in contaminated wheat.

The second part of Table 2 indicates the ability of the weevils to contaminate clean grain after removal from contaminated wheat. Weevils transmitted the organism continually to at least one of the three replicates of clean grain after being in the contaminated wheat 14 or 21 days. The longer periods in the contaminated wheat apparently aided the weevils either in picking up more bacteria or in retaining the bacteria longer. These tests also indicated that presence of S. montevideo inside the weevil does not always result in wheat contamination.

Table 2. Transmission of *Salmonella* by rice weevils (RW) from contaminated to clean wheat.

Length of time (days) weevils left in contaminated wheat	Presence of *Salmonella* externally (E) or in the digestive tract (DT) of RW						Presence of *Salmonella* in boxes of clean wheat after weevils transferred from contaminated wheat to clean wheat, then retransferred to new, clean wheat every 7 days. (Number represents transfer; letter represents replicate number													
	After RW transferred to clean wheat for 7 days				After RW transferred 5 times to boxes of clean wheat (7 days/box, 35 days total)		1			2			3			4			5		
	E	DT	E	DT	E	DT	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
7	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-						
14	+	+	+	-	+	+	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-
21	+	+	+	-	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-

Persistence of S. montevideo in Wheat
Infested with Rice Weevils

In the test for persistence of S. montevideo in wheat the number of live S. montevideo cells in the contaminated wheat 24 hours after inoculation was 2.7×10^6 cells/gm. After 21 days this dropped to 8.8×10^4 cells/gm. Rice weevils tested positive for S. montevideo externally and in the digestive tract throughout the 21-day period in both jars.

SUMMARY

Tests were conducted to determine the effect of the organism, Salmonella montevideo, on weevil longevity, number of adult progeny produced, and the length of time to first emergence of adult progeny. Survival of adults was greater in contaminated wheat than in the clean wheat. Rice weevils in the contaminated wheat had an average of 11 of 15 weevils/replicate alive after 11 weeks while after 8 weeks an average of only 10 weevils were alive in the clean wheat. The presence of S. montevideo did not adversely affect the adult weevils and may have been beneficial. The average number of progeny/female parent which emerged was higher in wheat contaminated with S. montevideo (133.7) than in the control (103.5). Differences

in the periods of time from placement of parent weevils in test media to first emergence of adult progeny were slight, but the periods for weevils reared in contaminated wheat were shorter and more consistent. Only 2 groups in contaminated wheat, compared to 5 in the control, took over 33 days from date of infestation to first emergence. When the wheat was inoculated with S. montevideo, the bacteria were in 5.0 ml solution which at least initially increased the moisture content of the wheat and perhaps was the cause for the insects' better performance in the contaminated wheat.

Development of an effective surface-sterilization technique included 70% ethyl alcohol followed by 1% mercuric chloride solution. Proteose peptone broth and brilliant green tetrathionate agar plates were used to indicate the incidence of S. montevideo.

Rice weevils retained the bacteria for at least 5 weeks after being in contaminated wheat for 14 and 21 days. The presence of S. montevideo in the digestive tract did not always result in contamination of clean wheat (6 of 30), but the possibility of contamination was greater when the weevils were in contaminated wheat for 14 and 21 days than when exposed only 7 days.

Tests were also conducted on the persistence of S. montevideo in wheat. Results indicated that the number of S. montevideo cells decreased rapidly from 2.7×10^6/gm to 8.8×10^4/gm during the first 21 days after inoculation. This decrease did not seem to affect the ability of rice weevils to pick up S. montevideo externally or internally.

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SALMONELLA MONTEVIDEO: ITS EFFECTS ON, AND TRANSMISSION BY, THE LESSER RICE WEEVIL (SITOPHILUS ORYZAE (L.)) IN WHEAT

by

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The potential role of stored-grain insects, particularly the lesser rice weevil, Sitophilus oryzae (L.), in transmission of Salmonella montevideo was explored. The effect of the organism, S. montevideo, on rice weevil longevity, number of adult progeny produced, and the length of time to first emergence of adult progeny was determined.

Adult weevils in S. montevideo-contaminated wheat had lower mortality rates than weevils in clean wheat. The average number of progeny per female parent which emerged was higher in the wheat with S. montevideo (133.7) than in clean wheat (103.5). Weevils in contaminated wheat underwent slightly shorter and more consistent average periods from the date that parent weevils were placed in the wheat until first emergence of adult progeny, compared to weevils in clean wheat. A possible cause for the weevils doing better in the contaminated wheat may have been due to the increased moisture added to the wheat when it was inoculated with the bacteria.

An effective technique for surface-sterilization of rice weevils and other stored-grain insects was achieved by using 70% ethyl alcohol followed by 1% mercuric chloride. Only weevils which were in contaminated wheat for at least 14 days tested positive for S. montevideo after being transferred

to clean wheat for 35 days. The length of time the weevils were exposed to the bacteria also affected the ability of the rice weevils to transmit S. montevideo to clean wheat. In another test, the rapid decrease of live S. montevideo cells over a 21-day period apparently did not affect the ability of the weevils to pick up S. montevideo externally or internally.