DISSEMINATION OF SALMONELLA MONTEVIDEO IN MASSES OF WHEAT BY THE RICE WEEVIL, SITOPHILUS ORYZAE (L.)

by

DAVID JAMES SCHUSTER

B. S., Kansas State University, 1970

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

Approved by:

Robert W. Mills
Major Professor

TABLE OF CONTENTS

INTRODUCTION

The rice weevil, Sitophilus oryzae (L.), is one of the most destructive insect pests of stored grain (Cotton, 1963). However, its role as a disseminator of microorganisms has not been fully studied. Husted et al. (1969) showed that weevils transferred Salmonella montevideo from a jar of contaminated wheat to one of clean wheat. It was the intention of this study to determine if the rice weevil could disseminate S. montevideo through wheat and to obtain data as to how far and in what numbers.

Salmonella montevideo accounted for 2 of 3 Salmonella isolations from grain in 1963 (U.S. Dept. of Health, Education and Welfare, 1964). Allred et al. reported in 1967 that this serotype led the list in the number of isolations from basic feed mills with 63 out of 544. In the United States from 1935 to 1963 it was one of the most prevalent serotypes isolated from humans (Galton et al., 1964). Salmonella montevideo was one of 6 serotypes responsible for 70-80% of all human cases in Canada from 1956-1960 (Bynoe and Yurack, 1964).

REVIEW OF LITERATURE

Insects have long been suspected disseminators of Salmonella. Salmonella enteritidis has been transmitted from houseflies to mice and from the mice back to flies (Ostrolenk and Welch, 1942). Greenberg (1964) succeeded in transmitting S. typhimurium from dog feces to human food by houseflies. After incubation the food was eaten by human volunteers who showed no disease symptoms but produced positive fecal samples.

Twelve Salmonella serotypes were recovered from flies associated with a Mexican slaughterhouse (Greenberg et al., 1963). Flies at the slaughterhouse

were mass-sprayed with uranin (sodium fluorescein dye) and then collected at sites as far away as 3 miles. From these, 7 of the 12 Salmonella serotypes were isolated (Greenberg and Bornstein, 1964).

Mackerras and Mackerras (1949) concluded that roaches were secondary infection sources during an infantile gastroenteritis epidemic in Queensland. Olson and Rueger (1950) decided from their studies that there were four ways roaches could transmit Salmonella: feces, vomitus, mouth contact during feeding and direct body contact.

During a small epidemic in Peiping, all body lice removed and examined tested positive for S. enteritidis. Some indication of internal multiplication was found by examining lice at intervals (Liu, Zia, and Chung, 1937). In 1943 Parker and Steinhaus found that S. enteritidis contaminated Rocky Mountain wood ticks could infect guinea pigs by their bite or their feces. Similar results were found for two common rat fleas (Esky, Prince, and Fuller, 1949).

Microorganisms and Stored Grain Pests

Grain mites, Acarus siro and Tyrophagus castellanii, were able to pick up storage fungi spores and inoculate clean grain. They carried the spores on their bodies, digestive tracts and feces (Griffiths, Hodson, and Christensen, 1959). Analogous results were obtained with the Angoumois grain moth (Misra, Christensen, and Hodson, 1961).

Sikorowski (1964) noted that the flat grain beetle, the red flour beetle and the saw-toothed grain beetle could carry a species of grain fungus to clean wheat. Further tests with the red flour beetle showed it was able to carry the organism internally.

Van Wyk, Hodson, and Christensen (1959) isolated more bacteria than fungi

from surface-sterilized larvae and adults of the confused flour beetle. As the insect population increased there was a decrease in storage fungi and an increase in bacteria. When this insect was reared simultaneously with the rice weevil, populations of bacteria and fungi increased but with fewer molds than when the rice weevil was grown alone.

Crawford, McDermott, and Musgrave (1960) isolated only three species of bacteria from three stages of development of the granary weevil (larva, pupa and adult): a Corynebacterium sp., a member of Bacillus cereus Frankland and Frankland group, and Micrococcus freudenreichii Guillebeau. Harein and De las Casas (1968) were able to isolate bacteria which included the Klebsiella-Aerobactor group, Escherichia intermidia, Micrococcus and Streptococcus spp., Proteus rettgeri, P. vulgaris, and Serratia marcescens. Bacterial counts from field collected weevils were always greater than laboratory-reared ones. Larvae, pupae and pre-emergence adults removed from wheat kernels were bacteria-free as were emerged adults held in sterile petri dishes.

Agrawal, Christensen, and Hodson (1957) showed that surface-sterilized grain containing granary weevils produced 10 times as many colonies of Aspergillus restrictus as did wheat with no insects. Fungi were present in the proventriculus, intestine and feces, and persisted in starved weevils until after their death. When Agrawal, Hodson, and Christensen (1958) introduced weevils into the bottom of a column filled with grain, storage fungi and moisture content increased in the same area where the insects did.

When a prepared suspension of lesser mealworm larvae or adults from the litter of a contaminated poultry house was injected into susceptible day-old chicks, 25 to 83% developed leukosis tumors (Eidson et al., 1966). Autoclaved suspension produced no tumors. Lesser mealworm adults collected in poultry brooder houses yielded 5 species of Salmonella at an incidence of 2.2%

(Harein et al., 1970). De las Casas, Pomeroy, and Harein (1968) fed lesser mealworm on dog food contaminated with S. typhimurium. While bacterial counts decreased 50% in the dog food, they increased from 1 to 2.4×10^6/insect.

Large numbers and multiple doses of Salmonella were required for even a few cells to be transmitted through the intestine of the hide beetle Dermestes maculatus (Julseth et al., 1969).

Some rice weevils exposed 7 days to wheat contaminated with about 10^6 S. montevideo/g were able to contaminate clean wheat for 7 days (Husted et al., 1969). Others exposed for 14 days contaminated clean wheat up to 28 days. Weevils put in contaminated wheat for 14 and 21 days and transferred weekly to clean wheat tested positive for S. montevideo externally and internally up to 5 transfers.

PRELIMINARY TESTS TO ESTABLISH TECHNIQUE

Materials & Methods

The rice weevil, Sitophilus oryzae (L.), is about 3 mm in length with head prolonged into a slender snout with mandibles at the end. The mated adult female chews a hole in a kernel, deposits an egg and seals the hole with a gelatinous material. The larval and pupal stages are completed inside the kernel in as few as 26 days. The adult chews its way out of the kernel and lives 4-5 months on the average (Cotton, 1963).

Weevil cultures were maintained in a dark room at about 27°C and 70% RH. To assure Salmonella-free insects, a sample of 50 weevils and 5 30 g samples of the hard red winter wheat to be used (each of which was withdrawn from a pint sample) were inoculated into Brilliant Green Tetrathionate Broth (Difco) and incubated at 37°C. After 24 and 48 hr, Brilliant Green Agar plates (Difco)

were streaked from each sample and incubated for 24 hr at 37°C. No Salmonella-like organisms were found so further identification was not necessary. Wheat for the weevil cultures and tests was withdrawn from sacks in the same lot as that tested above, equilibrated at about 13.65% moisture content and placed in a dark rearing room at about 27°C and 70% RH one week before being used (moisture determinations were done with Steinlite Model G and Motomco instruments).

Contaminated wheat for all experiments was prepared by inoculating yeast extract enriched proteose peptone agar slants from stock cultures of S. montevideo and incubating at 37°C for about 18 hr. The cells were washed off the slants with sterile 0.1% tryptone water. The turbidity of the suspension was standardized to approximately 200 times the Nephelometer #1 tube. One cc of suspension was added for each 100 g wheat to give a contamination of about 1×10^6 cells/g. After adding the suspension the wheat was rolled at least 10 min on a U.S. Stoneware Roller to distribute the S. montevideo throughout the wheat (20 g sample from each batch was tested which showed the contamination to be uniform among batches).

Test for Comparison of Three Sampling Techniques

Three metal drums (Plate I, d) approximately 21.6 cm diam by 24.2 cm deep were half-filled with wheat prepared as above. Placed in the center of each drum was a contamination cylinder (Plate I, e) about 5 cm diam by 7.8 cm deep of 3 mm hail screen into which was funneled a mixture of 120 g of wheat contaminated with 10^6 S. montevideo/g and 200 weevils (the hail screen permitted exit of the insects but retained the wheat). The weevils had been placed in the contaminated wheat, put in a dark rearing room at about 27°C and 70% RH for 24 hr, and then inactivated in a cooler (approximately 4°C) for 2 hr prior to

EXPLANATION OF PLATE I

Shows materials used in several of the tests:

a. Large drum 40 cm diam by 52.5 cm deep. Note Kelthane-treated filter papers taped over holes in lid.
b. Contamination cylinder (10.3 cm diam by 14.6 cm deep) of large drum (a).
c. Sample cage (5 cm diam by 7.5 cm deep) of large drum (a). Note qt fruit jar cap.
d. Small drum 21.6 cm diam by 24.2 cm deep. Note uncovered screened hole in lid and uncovered lateral probing holes.
e. Contamination cylinder (5 cm diam by 7.8 cm deep) for small drum (d).
f. Sample cage (3 cm diam by 5.5 cm deep) for small drum (d). Note aluminum cap.
g. Sack trier with 1.3 cm outside diameter.

THIS BOOK CONTAINS NUMEROUS PICTURES THAT ARE ATTACHED TO DOCUMENTS CROOKED.

THIS IS AS RECEIVED FROM CUSTOMER.

PLATE I

funneling. The drums were then filled to within 2.5 cm of the top, set in the rearing room for 2 wk and then removed to a freezer for one wk to kill the insects but destroy relatively few of the S. montevideo cells.

The first drum was sampled using a spoon sterilized with mercuric chloride between samples. The samples taken were: two surface samples about 5 cm from opposite drum edges, three 1.3 cm from the drum edge one on top of the other, one 2.5 cm from the center cage at the same level, and one immediately below the contamination cylinder (Plate II, Fig. 1). Another spoon was employed to scoop off layers of wheat until the desired sampling location had been reached. Each sample was poured into a sterilized white porcelain pan and insects removed with forceps to sterile test tubes where they were counted and macerated. Wheat samples were put in sterile pint fruit jars.

The second drum was sampled with cylindrical 3 mm hail screen cages (Plate I, f) about 3 cm diam by 5.5 cm deep capped with a 5 cm aluminum disk. These cages filled with wheat had been previously placed during filling at the same sample locations as in the first drum (Plate II, Fig. 2). It was assumed that weevils could move freely in and out of these cages. Wheat was spooned away from above the samples which were then lifted out with forceps and the wheat within treated the same as samples from the first drum.

The third drum (Plate II, Fig. 3) was sampled with a sack trier (Plate I, g) which had an outside diameter of 1.3 cm. One sample (C) was composed of three horizontal probes 1.3 cm from the bottom through a previously-cut hole covered with a sheet of plastic, a piece of metal and masking tape. One sample (B) was made up of four vertical probes 1.3 cm from the edge and another (A) of four vertical probes 2.5 cm from the contamination cylinder (X). The trier was sterilized between samples by rinsing three times with methanol and flaming each time. Samples were treated as for the other drums.

EXPLANATION OF PLATE II

Fig. 1. Spoon-sampled drum 21.6 cm by 24.2 cm deep. Letters A through G indicate locations sampled. "X" is the contamination cylinder. Scale is 1:4 in all illustrations.

Fig. 2. Cage-sampled drum showing all samples A through G.

Fig. 3. Probe-sampled drum in which sample C consisted of three probes and A and B of four probes each.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.

PLATE II

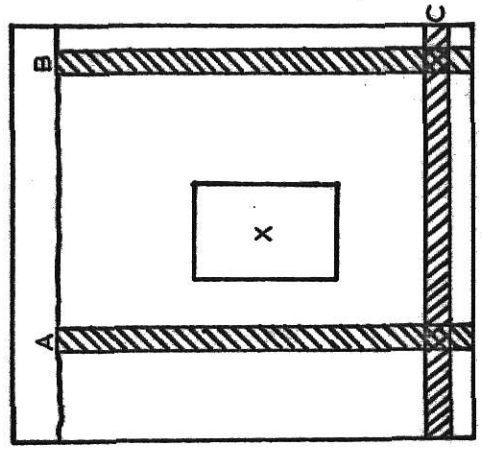

FIGURE 3

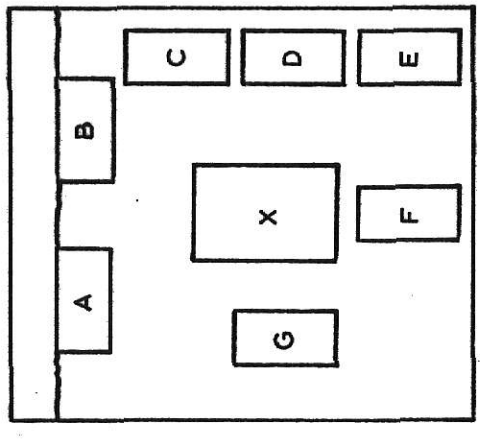

FIGURE 2

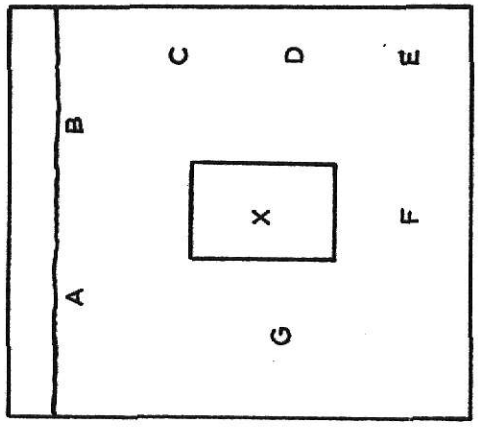

FIGURE 1

In all three drums the contamination cylinder and its insects constituted samples. Wheat and insect samples were individually inoculated with Brilliant Green Tetrathionate Broth and incubated at 37°C. After 24 and 48 hr, Brilliant Green Agar plates were streaked for each sample and incubated at 37°C for 24 hr. Two typical colonies were picked from each positive plate and streaked and stabbed into Triple Sugar Iron Agar slants (Difco) and incubated at 37°C for 24 hr. Cells from positive slants were mixed with poly H and O group C_1 antisera. Cells agglutinated in both antisera were considered S. montevideo.

Further Test of Probe Sampling Technique

Six drums were set up as in the last test except that before the test the insects were put on the contaminated wheat for 1-, 2- and 4-day periods rather than 24 hr. Two drums were assigned to each exposure period and held in a dark rearing room at 27°C and 70% RH for 2 wk and frozen at least 3 days.

One drum of each pair (Plate III, Fig. 1) had two lateral holes 6.5 cm and 21 cm from the top which were covered as those in the previous test. Four probes at the upper hole constituted one sample (B) and four at the lower another (C).

Two samples were taken from the other drum of each pair (Plate III, Fig. 2); one (C) consisted of four vertical probes as near to the drum edge as possible and the other (B) of four vertical probes 6.5 cm from the edge.

The trier was sterilized as in the previous test. The wheat and insects in the contamination cylinders (X) again constituted individual samples. All samples were treated as in the previous test for removing insects and determining S. montevideo presence.

EXPLANATION OF PLATE III

Fig. 1. First drum of a pair, in which samples B and C consisted of four probes at the location. "X" is the contamination cylinder (Scale 1:4).

Fig. 2. Second drum of a pair, in which samples B and C consisted of four probes at the location.

Fig. 3. One of five identical drums sampled by both cages (F, G, H, and I) and probe techniques. Samples B, C, D, and E each consisted of three probes at the location.

PLATE III

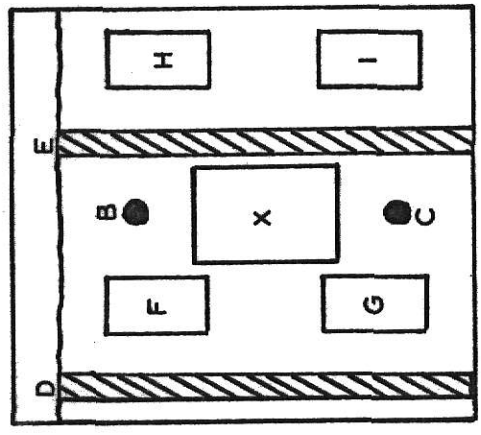

FIGURE 3

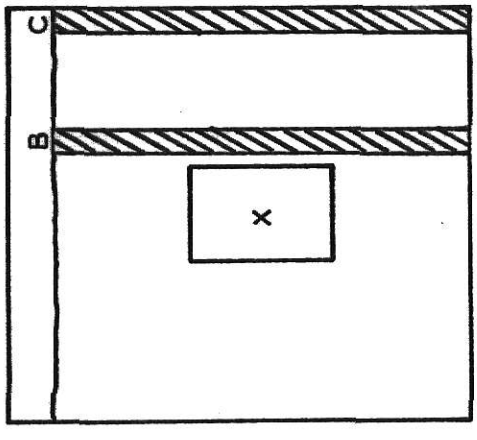

FIGURE 2

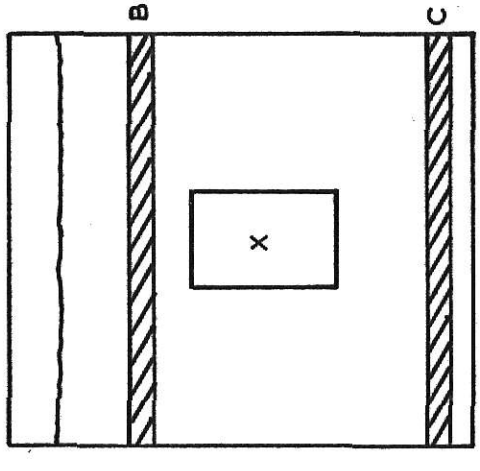

FIGURE 1

Test to Compare Cage and Probe Sampling Techniques

Five drums (Plate III, Fig. 3) were set up as before. The weevils were put on the contaminated wheat and placed in a dark rearing room at 27°C and 70% RH 4 days before the test was started. Four cylinders about 3 cm diam by 5.5 cm deep of 3 mm hail screen, capped with 5 cm diam aluminum lids and partially filled with 25 g wheat were placed in each drum during filling (the cages were only 4/5 filled to permit the insects more ease of entrance and exit). Two (I & G) were set 2.5 cm from the bottom and 1.3 cm and 6.3 cm from the sides. Each drum had two lateral holes--2.5 cm above and 2.5 cm below the contamination cylinder (X). They were covered as before. Sixty-mesh/2.5 cm screen was soldered over a 5 cm diam hole in each drum lid and covered with Kelthane-impregnated filter paper taped into position to eliminate the build-up of CO_2, depletion of O_2, and possible infestation of mites. The drums were then put in the rearing room for 2 wk after which they were frozen for at least 3 days. The same trier and sterilizing method were used as in previous tests. One sample (D) consisted of three vertical probes 1.3 cm from the drum edge and another sample (E) of three probes 6.3 cm from the edge. The upper lateral hole was horizontally probed three times to form one sample (B) and the lower hole three times to form another (C).

After probing, the wire cages were removed by carefully scooping wheat from above until they could be removed with forceps. The contamination cylinders were again treated as samples. All wheat samples were treated as before to separate insects and determine S. montevideo presence.

Results & Discussion

Test for Comparison of Three Sampling Techniques

Wheat and insects recovered from the contamination cylinders (X) were all positive (Table 1). Spooned wheat samples from drum #1 (Plate II, Fig. 1) were all positive. These results were questionable since insects from only one sample of four which contained weevils were positive. It was believed this technique allowed positive wheat to shift and "sift" to different locations.

In drum #2 (Plate II, Fig. 2) only sample F immediately below the contamination cylinder was positive and insects retrieved from two other samples were negative.

Samples A and B, 2.5 and 5 cm from the contamination cylinder, were positive for both the wheat and insects in drum #3 (Plate II, Fig. 3). Sample C, 1.25 cm from the bottom, was negative. This technique was the most consistent and was selected for further testing. Insects were so consistently negative in all drums that longer exposure periods were tried in the next test.

Further Test of Probe Sampling Technique

Wheat and insects from the contamination cylinders (X) were all positive (Table 2). Wheat samples (B) from the 1-day exposure drums were positive only up to 2.5 cm from the top and 1.25 cm from the side of the contamination cylinder (Plate III, Figs. 1 & 2). Insects from these and further removed samples were negative. In the 2-day and 4-day exposure drums positive wheat samples (B & C) were recovered up to 5 cm from the bottom and 6.25 cm from the side of the contamination cylinder. Insects from these and other samples were positive in 3 out of 5 cases. These results indicated that the

Table 1. Comparison of three sampling techniques to determine the ability of the rice weevil to transfer Salmonella montevideo into clean wheat after exposure to contaminated wheat (see Plate II).

		Samples							
		A	B	C	D	E	F	G	X
	Wheat + or -	+	+	+	+	+	+	+	+
Drum #1* Spoon-sampled	No. insects in sample	4	1	0	0	0	2	1	9
	Insects + or -	+	-				-	-	+
	Wheat + or -	-	-	-	-	-	+	-	+
Drum #2 Cage-sampled	No. insects in sample	3	0	1	0	0	0	0	18
	Insects + or -	-		-					+
	Wheat + or -	+	+	-	**				+
Drum #3 Probe-sampled	No. insects in sample	1	1	1					10
	Insects + or -	+	+	-					+

* 50 weevils taken from the top of the wheat surface were positive.
** Were no samples D through G (Plate II, Fig. 3).

Table 2. Further test of probe sampling technique for determination of ability of rice weevils to transfer Salmonella montevideo into clean wheat after 1-, 2-, or 4-day exposures in contaminated wheat. Drums #1, 3, & 5 (Plate III, Fig. 1) were horizontally probed and drums #2, 4, & 6 (Plate III, Fig. 2) vertically.

Exposure period			Samples X	B	C
1-day	Drum #1	Wheat(+ or -)	+	+	-
		No. of insects	33	2	3
		Insects(+ or -)	+	-	-
	Drum #2	Wheat(+ or-)	+	+	-
		No. of insects	39	1	0
		Insects(+ or -)	+	-	
2-day	Drum #3	Wheat(+ or -)	+	+	+
		No. of insects	15	3	1
		Insects(+ or -)	+	+	+
	Drum #4	Wheat(+ or -)	+	-	-
		No. of insects	37	4	0
		Insects(+ or -)	+	-	
4-day	Drum #5	Wheat(+ or -)	+	-	-
		No. of insects	29	5	0
		Insects(+ or -)	+	-	
	Drum #6	Wheat(+ or -)	+	+	+
		No. of insects	27	1	0
		Insects(+ or -)	+	+	

longer exposure periods were more satisfactory for later tests.

Test to Compare Cage and Probe Sampling Techniques

Wheat and insects from all the contamination cylinders (X) were positive (Table 3; Plate III, Fig. 3). Only four other wheat samples were positive at distances up to 2.5 cm from the contamination cylinder for probe sampling and up to .6 cm for the cages. More insects were consistently recovered from cage samples than from probed samples so this method was used in subsequent tests. Insects were all negative except for those from the contamination cylinders so the S. montevideo dosage was increased from 10^6 to 10^7 cells/g wheat in later tests.

EXPERIMENTAL TESTS

Material & Methods

Drum Test

A metal drum (Plate I, a) approximately 40 cm diam by 52.5 cm deep was filled with wheat to within 5 cm of the top (Plate IV). Placed in the center during the filling was a contamination cylinder (Plate I, b) 10.3 cm diam by 14.6 cm deep of 3 mm hail screen into which was funneled 1000g of wheat containing 10^7 S. montevideo/g and 2000 unsexed rice weevils. The insects had been placed on the contaminated wheat 1 wk before the test and held in a dark rearing room at 27°C and 70% RH. Two hr before funneling, the wheat and weevils were placed in a cooler at 4°C. Twenty seven sampling cylinders (Plate 1,c) of 3 mm hail screen about 5 cm diam by 7.5 cm deep and capped with quart fruit jar disk lids were 4/5 filled with 100 g of wheat and positioned 1.25, 2.5, 3.75, 5.0, 6.26, 7.5, and 17.5 cm from the drum edge in four spiral

Table 3. Comparison of cage and probe sampling techniques for determination of ability of the rice weevil to transfer Salmonella montevideo from contaminated to clean wheat (see Plate III, Fig. 3).

		Samples								
		*	Probe				Cage			
		X	B	C	D	E	F	G	H	I
Drum #1	Wheat(+ or -)	+	-	-	-	-	-	+	-	-
	No. of insects	28	0	0	0	2	5	2	1	0
	Insects(+ or -)	+				-	-	-	-	
Drum #2	Wheat(+ or -)	+	-	-	-	-	-	-	-	-
	No. of insects	32	3	0	1	1	10	2	3	0
	Insects(+ or -)	+	-		-	-	-	-	-	
Drum #3	Wheat(+ or -)	+	-	+	-	-	-	+	-	-
	No. of insects	35	1	0	1	0	0	1	6	1
	Insects(+ or -)	+	-		-			-	-	-
Drum #4	Wheat(+ or -)	+	-	-	-	-	-	-	-	-
	No. of insects	14	4	0	0	1	1	1	1	0
	Insects(+ or -)	+	-			-	-	-	-	
Drum #5	Wheat(+ or -)	+	+	-	-	-	-	-	-	-
	No. of insects	28	0	0	3	0	1	0	0	2
	Insects(+ or -)	+			-		-			-

* Contamination cylinder.

EXPLANATION OF PLATE IV

Fig. 1. Metal drums 40 cm diam by 52.5 cm deep (Scale 1:4). Surface cage samples (crossectional view) are 1, 2, 3, and 4. Layers 1 through 4 show levels at which cage samples were spirally arranged (Fig. 2). Samples G3 and G4 were depressed in relation to their respective layers to fit under the contamination cylinder (X).

Fig. 2. Shows one spiral layer superimposed over the contamination cylinder (X). Each layer of Fig. 1 contained one of these spiral arrangements. Layer 2 of Fig. 1 had no "G" sample.

PLATE IV

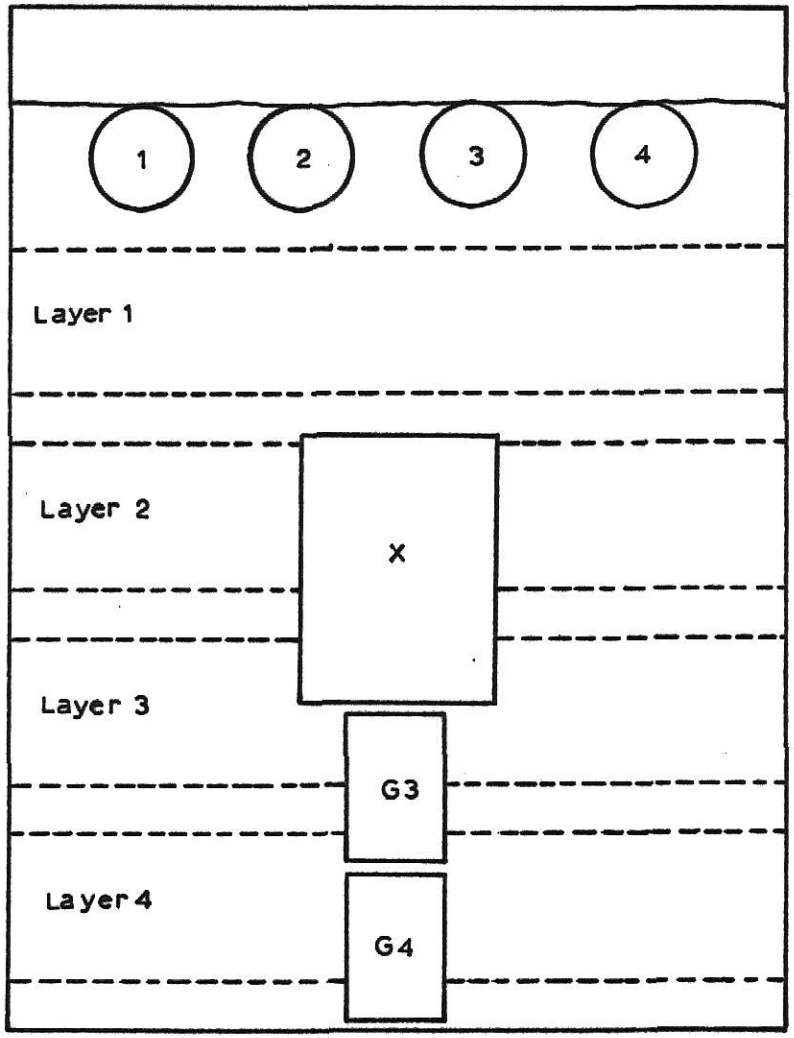

FIG. 1

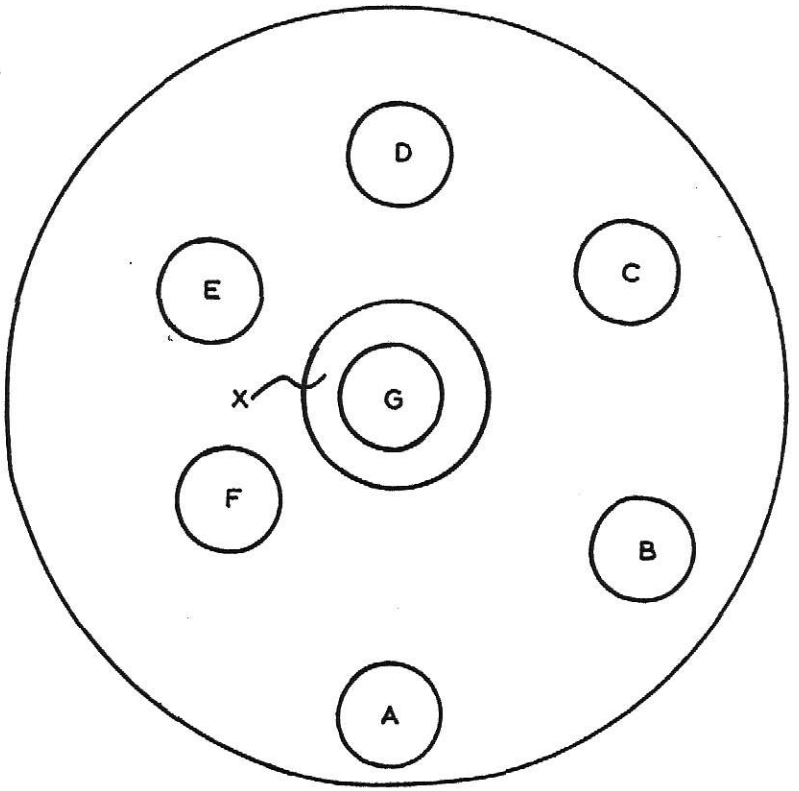

FIG. 2

layers separated by 2.5 cm. Samples 17.5 cm from the edge in layers 3 and 4 were depressed (G3 and G4) to fit under the contamination cylinder. The contamination cylinder (X) constituted another sample and was substituted for sample G of layer 2. Four sample cages were placed horizontally just under the surface and were separated from each other and the drum edge by 3.75 cm. Sixty-mesh/2.5 cm screen was soldered over two 5 cm diam holes in the drum lid and covered with Kelthane-treated filter papers taped into position. Each of three replicates were left 1 wk in a dark rearing room at 27°C and 70% RH and frozen 1 wk (weevil age from egg deposition to freezing ranged from 58 to 70 days).

Wheat was scooped away from the top of the sample cages which were withdrawn by forceps and treated as in the preliminary tests to separate insects and determine S. montevideo presence.

A control drum was identical to the above except no weevils were introduced.

Wooden Troughs--Two Contamination Levels

Howe (1951) showed that, when weevils were placed in one end of a horizontal tower 10.2 cm diam by 132.1 cm and left for one week, some individuals reached the end. Following this type of approach, two wooden troughs 1.5 m by 6.25 cm by 6.25 cm inside measurements were constructed of 1.9 cm white pine (Plate V, Fig. 1). A hole 5 cm diam was made in each at one end, covered with 60-mesh screen cemented with epoxy, and covered with a Kelthane-treated filter paper taped into position. At the opposite end of each trough was a 5 cm by 6.25 cm by 6.25 cm contamination chamber screened (3 mm hail screen) on the side facing the inside of the trough. Each trough was divided into two

EXPLANATION OF PLATE V

Fig. 1. Shows disassembled and empty wooden trough apparatus. Contamination box is labled X, and A through O are the cylindrical sample cages.

Fig. 2. Shows disassembled and empty elongate metal chamber. Numerals denote chamber sections and letters samples (X was the contamination box). Samples A, C, E, G, and I were large to permit enumeration of S. montevideo and samples B, D, F, H, and J were small. Note sample cages and bent metal covers for each sample location.

PLATE V

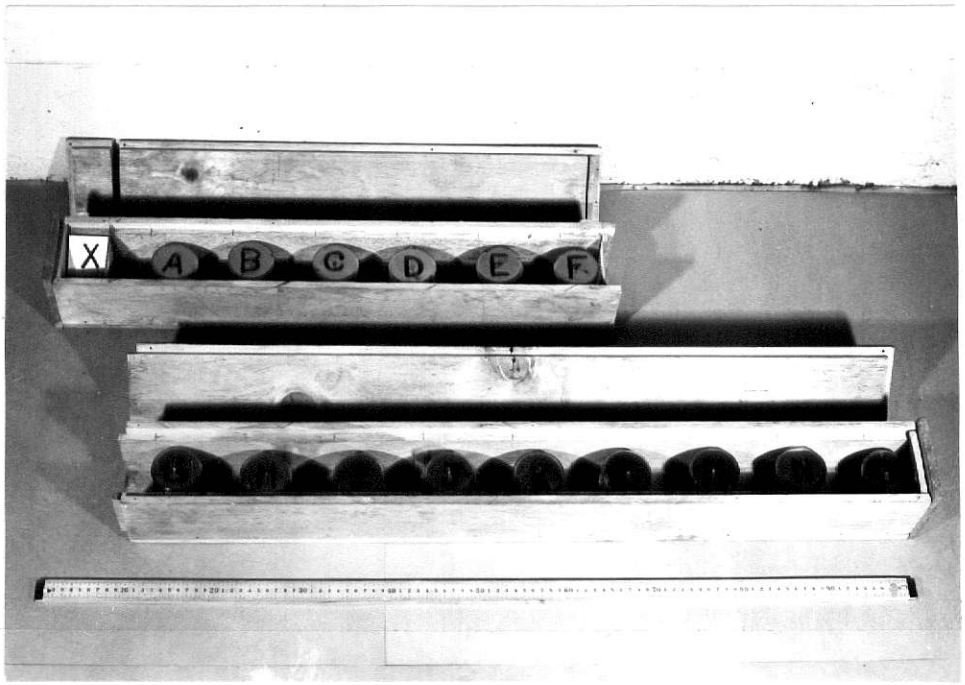

Fig. 1

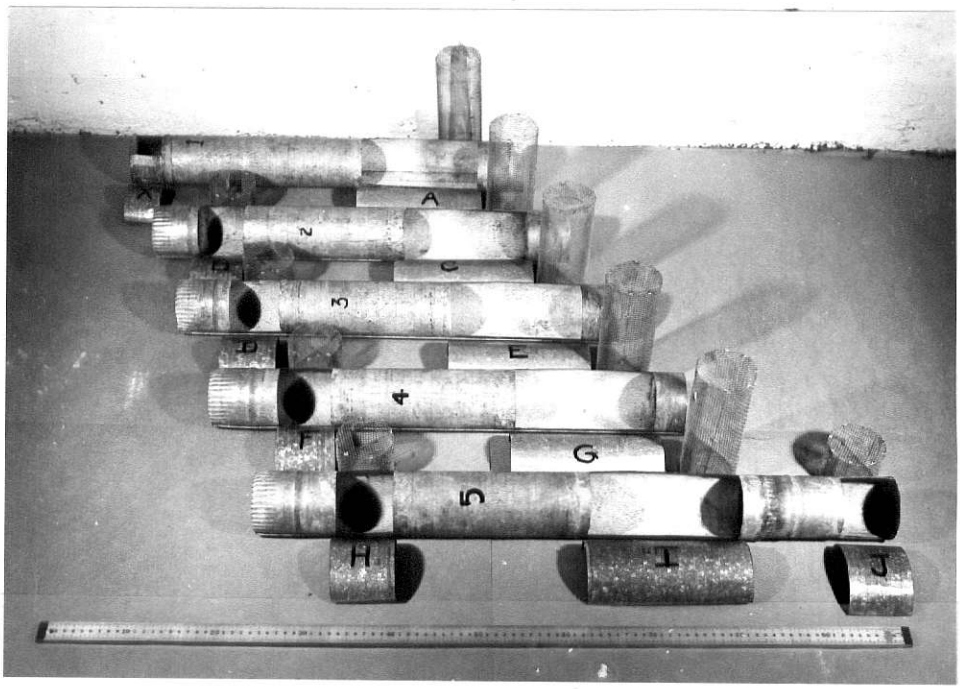

Fig. 2

portions (one of which was screened at the point of separation with 3 mm hail screen which permitted passage of insects) to permit autoclaving after tests. All wood joints were double half-mortise. Seventy grams of wheat were placed in each of 15 cylindrical cages about 5 cm by 5 cm made of 3 mm hail screen with 5.6 cm diam metal lids and placed approximately 5 cm from the contamination chamber and from each other throughout the length of the trough. Wheat was then poured, but not packed, to fill the trough and the wooden lids screwed into place. All joints were taped with 5 cm masking tape. One week before the test was begun, 200 weevils were put in each of 2 100 g portions of wheat, one of which was contaminated with 10^4 S. montevideo/g and the other 10^7 S. montevideo/g, and placed in a dark rearing room at 27°C and 70% RH. Insects were immobilized by putting the samples in a 4°C cooler for 2 hr. Each of the contaminated and infested samples was funneled into the contamination chambers of separate troughs. The lids of the boxes were screwed and taped into position and the troughs put into the rearing room for 1 wk after which they were frozen for 3 days (insect ages from egg deposition to freezing were 42-49 days).

After freezing the lids were removed and the contents of the contamination chamber and sample cages treated as in previous tests to determine S. montevideo presence on wheat and insects.

Wooden Trough--One Contamination Level

It was decided in the preceding test that only 10^7 S. montevideo/g would be used. One wooden trough (Plate V, Fig. 1) was set up and sampled identically to those above with the exception that the contaminated wheat contained 10^7 S. montevideo/g (insect age from egg deposition to freezing ranged from 49 to 61 days). This was replicated three times over an 8 wk period. A

control trough was also run using 10^7 S. montevideo/g but no insects.

Elongate Metal Chamber

A 311.25 cm long test chamber (Plate V, Fig. 2) was constructed from 6 60 cm long sections of 7.5 cm galvanized steel stove pipe. The third and fifth sections (plus 18.5 cm of the sixth soldered to it) had one end screened with 3 mm hail screen which permitted passage of insects. The opposite end of the fifth was solder-sealed with a disk of galvanized steel, which had a 4.5 cm diam hole with 60-mesh/2.5 cm screen soldered and a Kelthane-treated filter paper taped over it, and that of the third remained open. Sections two and four had both ends screened. These steps allowed the chamber to be disassembled for freezing and autoclaving. A 5 cm end portion of section one was screened off to form an initial contamination and infestation box (the opposite end was soldered-sealed with a galvanized steel disk). Beginning with the larger, alternating 17.5 cm long by 10 cm and 6.6 cm long 10 cm holes were cut every 30 cm from the top of the chamber. Using 3 mm hail screen, five cylindrical sample cages 6.9 cm diam by 17.8 cm long and five cages 6.9 cm diam by 6.25 cm long were constructed. The larger samples were for later enumeration of S. montevideo on the wheat. One end of each cage had a 3.1 cm square flap through which was funneled 560 g wheat in the larger and 170 g in the smaller cages. Starting with number one, each section of the chamber was held vertically and filled with wheat up to the first hole. An appropriately sized filled sample cage was inserted in the hole which was then covered with a bent piece of galvanized steel lined with an adhesive backed rubberized cork tape. This same procedure was followed until all holes in the five sections had been filled. The total trough was then assembled by slipping each section into the preceding one to a predetermined mark and then sealing with several layers of

masking tape.

One week before the test was begun, 1000 weevils were put on 180 g of wheat contaminated with 10^7 S. montevideo/g and set in a dark rearing room at 27°C and 70% RH. The wheat and insects were put in a 4°C cooler 2 hr and then funneled into the contamination box of the chamber and sealed as above. The completed chamber was put in the rearing room for 1 wk at which time it was separated in two places above a pan containing mercuric chloride (to catch falling wheat and insects), the open ends sealed with masking tape and the sections placed in a freezer for 3 days. After freezing the sampling holes were carefully opened and the smaller sample cages withdrawn and treated as in previous tests. The larger samples were withdrawn and the insects separated as in other tests and the wheat divided into 5 100 g aliquots (into qt fruit jars), 5 10 g aliquots (into pt fruit jars) and 5 1 g aliquots (into large test tubes). These steps were taken to determine the number of S. montevideo present by the Most Probable Numbers method (Galton, Morris, and Martin, 1968). The insect and wheat samples were then cultured as in previous tests. A total of three replicates were completed.

Results & Discussion

Drum Test

All wheat and insect samples from the contamination cylinders (X) were positive (Table 4; Plate IV). All other insect samples were positive except for one, thus showing that the insects retained the S. montevideo during the test period. Wheat samples from the surface and top three layers were all positive. Salmonella montevideo incidence in the bottom layer decreased toward the edge of the drum. Insect recovery was also lowest in the bottom

Table 4. Test with metal drum (40 cm diam, 52.5 cm deep) to determine ability of the rice weevil to transfer *Salmonella montevideo* from contaminated to clean wheat.

Sample*	No. of reps. with wheat +	Ave. no. of insects/rep.	No. of reps. with insects	No. of reps. with insects +	Control wheat(+ or -)
1	3	4 1/3	2	2	-
2	3	3 1/3	3	3	-
3	3	5 1/3	3	3	-
4	3	6 2/3	2	2	-
A1	3	2 2/3	3	2	-
B1	3	3 2/3	2	2	-
C1	3	4 2/3	3	3	-
D1	3	8 2/3	3	3	-
E1	3	12 2/3	3	3	-
F1	3	15 2/3	3	3	-
G1	3	31 1/3	3	3	-
A2	3	5	3	3	-
B2	3	7 2/3	3	3	-
C2	3	10 2/3	3	3	-
D2	3	20 2/3	3	3	-
E2	3	24 2/3	3	3	-
F2	3	29 2/3	3	3	-
A3	3	3	2	2	-
B3	3	1 2/3	2	2	-
C3	3	6	3	3	-
D3	3	9	3	3	-
E3	3	19	3	3	-
F3	3	35	3	3	-
G3	3	60 1/3	3	3	-
A4	1	2/3	1	1	-
B4	1	0	0	0	-
C4	2	0	0	0	-
D4	1	1/3	1	1	-
E4	2	1/3	1	1	-
F4	2	1/3	1	1	-
G4	3	1/3	1	1	-
X	3	50+	3	3	+

* Refer to Plate IV. For samples A1, B1, etc., the letters refer to locations of samples in relation to the contamination cylinder in the horizontal plane; numbers refer to the "layer" or depth of the spiral of samples.

layer. The recovery of more positive samples where more insects were found suggested that the insects were transporting the bacteria. This was substantiated by the fact that an insect-free control produced no positive wheat samples. The increase from 4 to 7 days exposure period and increase from 10^6 to 10^7 S. montevideo/g wheat over previous tests appeared to increase the dissemination capability of the rice weevil.

Since the insects were able to carry S. *montevideo* to every sampling area in the drum (max. 12.5 cm), further tests were done to determine if dissemination could occur over longer distances through wheat.

Wooden Troughs--Two Contamination Levels

The only positive sample from the trough with 10^4 S. montevideo/g in the contamination box was the wheat from the contamination box (Table 5; Plate V, Fig. 1). Insect samples were all negative.

Wheat and insects were positive from the contamination box in the trough using 10^7 S. montevideo/g. Another wheat sample was positive at 5 cm from the contamination box while positive insects were recovered from as far away as 55 cm. This contamination level was used in the remaining tests.

Wooden Trough--One Contamination Level

Wheat and insects were positive in all three replicates from the contamination boxes and up to 75 cm from the contamination boxes (Table 6; Plate V, Fig. 1). Every wheat sample was positive from one trough, but the other troughs were intermittent after 75 cm. Weevils were able to transmit S. montevideo the full distance of the trough (1.5 m) in two cases. The control was completely negative except for the contamination box.

Table 5. Wooden trough tests to determine the ability of the rice weevil to transfer Salmonella montevideo from wheat contaminated at two levels (10^4 and 10^7 S. montevideo/g in contamination box X) to clean wheat (Plate V).

Sample & Distance*		Contamination Level					
		10^4 cells/g			10^7 cells/g		
		Wheat (+ or -)	No. of Insects	Insects (+ or -)	Wheat (+ or -)	No. of Insects	Insects (+ or -)
X	0 cm	+	4	-	+	4	+
A	5	-	22	-	+	20	+
B	15	-	22	-	-	17	+
C	25	-	10	-	-	11	+
D	35	-	8	-	-	13	-
E	45	-	7	-	-	2	-
F	55	-	3	-	-	11	+
G	65	-	3	-	-	3	-
H	75	-	1	-	-	0	
I	85	-	0		-	0	
J	95	-	6	-	-	3	-
K	105	-	0		-	1	-
L	115	-	2	-	-	0	
M	125	-	2	-	-	1	-
N	135	-	0		-	3	-
O	145	-	5	-	-	1	-

* Distance in cm from the contamination box.

Table 6. Replicated wooden trough test to determine the ability of the rice weevil to transfer *Salmonella montevideo* from wheat contaminated with 10^7 *S. montevideo*/g to clean wheat (see Plate V).

Sample & Distance*		Replicate #1			Replicate #2			Replicate #3			Control
		Wheat (+ or -)	No. of Insects	Insects (+ or -)	Wheat (+ or -)	No. of Insects	Insects (+ or -)	Wheat (+ or -)	No. of Insects	Insects (+ or -)	Wheat (+ or -)
X	0cm	+	13	+	+	3	+	+	6	+	+
A	5	+	13	+	+	14	+	+	22	+	-
B	15	+	11	+	+	15	+	+	13	+	-
C	25	+	5	+	+	7	+	+	11	+	-
D	35	+	9	+	+	14	+	+	12	+	-
E	45	+	5	+	+	10	+	+	13	+	-
F	55	+	10	+	+	18	+	+	5	+	-
G	65	+	4	+	+	4	+	+	1	+	-
H	75	+	4	+	+	4	+	+	1	+	-
I	85	+	3	+	+	1	-	-	2	+	-
J	95	-	1	+	+	1	+	-	1	+	-
K	105	+	0		+	2	+	+	0		-
L	115	+	2	+	+	0		-	2	+	-
M	125	-	1	+	+	1	+	-	1	+	-
N	135	-	0		+	8	-	-	0		-
O	145	+	9	+	+	5	+	-	1	+	-

* Distance in cm from the contamination box.

Elongate Metal Chamber

A metal test chamber (Plate V, Fig. 2) was constructed for these tests to eliminate any effects wood resins might have on the S. montevideo. One thousand weevils were used instead of 200 to try and increase the maximum distance of transmission. Wheat recovered from the contamination boxes of all three replicates was positive while insects were positive in only two (Table 7). All other insect samples from the first two replicates were positive at distances from 210 cm to 270 cm from the contamination box. Only 3 of 8 insect samples in the last replicate were positive (C, E, and F) and only one colony was recovered from a plate streaked from one of these (C). No explanation is offered.

The wheat samples in the smaller cages (B, D, F, H, and J) were positive at least 120 cm from the contamination box in all replicates and up to 180 cm in one. The larger samples (A, C, E, G, and I) had aliquots positive as far as 270 cm in two replicates. Enumeration results showed that S. montevideo was present in small numbers even at a distance of only 30 cm from the contamination box (.92 to >1.60 cells/g). Between 30 and 90 cm there was a rapid drop beyond which recoveries leveled off ranging from .013 to .000 cells/g at distances of 150 to 270 cm.

The significance of these results is difficult to assess. Thomas and Hobson (1955) showed that coli-aerogenes bacteria were present in 73% of the ears and panicles of growing cereals tested and that their numbers increased during the growing season. Crumrine and Foltz (1969) showed that S. montevideo could survive at least 28 wk on wheat held at moisture contents comparable to those in commercially binned wheat. Animals could conceivably contaminate wheat in the field or in the bin or anywhere between and the wheat remain infective for relatively long periods of time.

The low numbers of S. montevideo shown to be transmitted in this study are

Table 7. Elongate metal chamber test to determine how far and in what numbers the rice weevil could transfer Salmonella montevideo from contaminated wheat (10^7 cells/g) to clean wheat (see Plate V).

		Samples & Distances from Contamination Chamber in cm										
		X	A	B	C	D	E	F	G	H	I	J
			30	60	90	120	150	180	210	240	270	300
	Wheat(+ or -)	+	100g 5+*	+	100g 2+	+	100g 1+	-	100g 0+	-	100g 1+	-
			10g 5+		10g 1+		10g 1+		10g 0+		10g 1+	
			1g 3+		1g 0+		1g 0+		1g 0+		1g 0+	
Rep. #1	No. of S. montevideo/g		.920		.007		.004		.000		.004	
	No. of insects	105	156	18	20	9	9	1	7	0	0	0
	Insects(+ or -)	+	+	+	+	+	+	+	+			
	Wheat(+ or -)	+	100g 5+	+	100g 5+	+	100g 0+	-	100g 1+	-	100g 0+	-
			10g 5+		10g 4+		10g 0+		10g 1+		10g 0+	
			1g 4+		1g 2+		1g 0+		1g 0+		1g 0+	
Rep. #2	No. of S. montevideo/g		1.600		.220		.000		.004		.000	
	No. of insects	152	197	50	55	8	12	2	5	0	3	0
	Insects(+ or -)	+	+	+	+	+	+	+	+		+	
	Wheat(+ or -)	+	100g 5+	+	100g 5+	+	100g 3+	+	100g 0+	-	100g 0+	-
			10g 5+		10g 5+		10g 0+		10g 0+		10g 0+	
			1g 5+		1g 2+		1g 2+		1g 0+		1g 1+	
Rep. #3	No. of S. montevideo/g		>1.600		.540		.013		.000		.002	
	No. of insects	43	196	40	52	2	15	1	2	0	0	0
	Insects(+ or -)	-	-	-	+	-	-	+	+			

* Number of positive subsamples (of 5) of each size.

not very significant from a human health standpoint since larger dosages are normally required to produce disease symptoms in adults. Theoretically, though, one surviving organism is capable of causing disease. Also, rats have been shown to excrete positive fecal pellets after ingesting 1 to 15 Salmonella and were able to contaminate other rats in their colony (Welch, Ostrolenk, and Bartram, 1941). They could, therefore, ingest the low numbers of Salmonella organisms that insects might transmit, increase the numbers of bacteria in their digestive tracts, and recontaminate the wheat with their feces. The same situation might exist with other animals, particularly birds.

At first glance the distance results might seem more dramatic. If rice weevils in the field could carry Salmonella 2.7 m in any direction from the source, they would be capable of contaminating all parts of a cylindrical bin of wheat with a volume of 127.7 m^3 or 3,547.2 bu. Couple this with the possible recontamination by animals as mentioned above and the amount of wheat contaminated could be increased. However, the conditions of these tests and those encountered in the field are quite different. In the tests, large numbers of weevils per gram of wheat were exposed to highly contaminated wheat for relatively long periods of time. In a natural situation a weevil population approaching those used in these tests would result in near destruction of the grain. The lower field population would likewise decrease the dispersal pressure on the insects. Weevils would not be confined and, hence, their exposure to a concentration of contaminated wheat would likely be short--in some cases as little as the time necessary to move through it. Their freedom to move in a much larger grain mass would also result in fewer insects recoverable at increased distances, thus lessening the possibility of Salmonella being deposited.

Further tests following the same scale as these but with varying Salmonella

contaminations and weevil populations would be unwarranted. Repetition of these tests using different stored products insects might prove useful. Employing the rice weevil and applying these same techniques to bin-size situations would be valuable but impractical. Not only would the health of the handlers and samplers be in jeopardy, but escaping weevils might contaminate nearby grain bins, feed rooms, etc. Hermetic storage to contain the insects would only partially solve the problem while imposing bin conditions not commonly found. These questions demonstrate that while the rice weevil probably plays a relatively small role in the cycle of Salmonellosis, it should not be overlooked.

SUMMARY

The ability of the rice weevil, Sitophilus oryzae (L.), to disseminate Salmonella montevideo through wheat was studied. Tests indicated how far and in what numbers the organisms were transported.

Preliminary tests were conducted to establish a sampling technique and exposure period. Insects exposed to contaminated wheat for various lengths of time were introduced into the centers of small grain masses in metal drums about 21.6 cm diam by 24.2 cm deep which were sampled by three methods. Cylindrical cages of 3 mm hail screen were considered the most convenient and consistent sampling means. Results also indicated an exposure period of 1 wk to be best. Brilliant Green Tetrathionate Broth (Difco), Brilliant Green Agar plates (Difco), Triple Sugar Iron Agar slants (Difco), and poly H and O group C_1 antisera were used in that order to determine the presence of S. montevideo in these and later tests.

A larger grain mass in a metal drum 40 cm diam by 52.5 cm deep was centrally infested with insects previously exposed 1 wk to wheat contaminated with 10^7

S. montevideo/g. Three replicates and an insect-free control were completed. The number of positive wheat samples was lowest where the number and frequency of insects was lowest while control wheat samples were all negative. The rice weevil was assumed to be transporting the organism.

One 1.5 m long by 6.25 cm by 6.25 cm wooden trough filled with wheat was infested at one end with weevils exposed to wheat contaminated with 10^4 S. montevideo/g and another similarly infested with weevils exposed to 10^7 S. montevideo/g. Both were sampled with 5 cm by 5 cm cylindrical cages of 3 mm hail screen filled with wheat and spaced 5 cm from each other throughout the length of the trough. Wheat was positive in the former trough only from the contamination box while no insects were positive. Wheat was positive from the latter trough 5 cm from the contamination box while insects were positive up to 55 cm. Three replicates as above were each infested at one end with insects exposed 1 wk to wheat contaminated with 10^7 S. montevideo/g. An insect-free control was also completed. Every wheat sample was positive from one replicate, but the other two were intermittent beyond 75 cm from the contamination point. Weevils were able to transport S. montevideo the full distance of the trough in two replicates. The control wheat samples were negative.

A cylindrical metal chamber 311.25 cm long and 7.5 cm diam was filled with wheat and infested at one end with weevils previously exposed 1 wk to wheat contaminated with 10^7 S. montevideo/g. Three replicates were completed. Positive wheat was recovered at least 120 cm from the infestation point in all replicates and as far away as 270 cm in two. Enumeration results showed that S. montevideo was present in small numbers, even at a distance of only 30 cm from the infestation point (.920 to >1.600 cells/g). Between 30 and 90 cm there was a rapid drop beyond which recoveries leveled off ranging from .013 to .000 cells/g at distances of 150 to 270 cm.

LITERATURE CITED

Agrawal, N. S., C. M. Christensen, and A. C. Hodson. 1957. Grain storage fungi associated with the granary weevil. J. Econ. Entomol. 50:659-663.

Agrawal, N. S., A. C. Hodson, and C. M. Christensen. 1958. Development of granary weevils and fungi in columns of wheat. J. Econ. Entomol. 51:701-702.

Allred, J. N., J. W. Walker, V. C. Beal, and F. W. Germaine. 1967. A survey to determine the Salmonella contamination rate in livestock and poultry feeds. J. Amer. Vet. Med. Ass. 151:1857-1860.

Bynoe, E. T., and J. A. Yurack. 1964. Salmonellosis in Canada. In The world problem of salmonellosis. E. Van Oye, Ed. Dr. Junk Publishers--The Hague, Brussels. 606 pp.

Cotton, R. T. 1963. Pests of stored grain and grain products. Burgess Pub. Co., Minn. 318 pp.

Crawford, R. E., L. A. McDermott, and A. J. Musgrave. 1960. Microbial isolations from the granary weevil Sitophilus granarius (L.) (Coleoptera: Curculionidae). Can. Entomol. 92:577-581.

Crumrine, M. H., and V. D. Foltz. 1969. Survival of Salmonella montevideo on wheat stored at constant relative humidity. Appl. Microbiol. 18:911-914.

De las Casas, E., B. S. Pomeroy, and P. K. Harein. 1968. Infection and quantitative recovery of Salmonella typhimurium and Escherichia coli from within the lesser mealworm, Alphitobius diaperinus (Panzer). Poultry Sci. 47:1871-1875.

Eidson, C. S., S. C. Schmittle, R. B. Goode, and J. B. Lal. 1966. Induction of leukosis tumors with the beetle Alphitobius diaperinus. Amer. J. Vet. Res. 27:1053-1057.

Esky, C. R., F. M. Prince, and F. B. Fuller. 1949. Transmission of Salmonella enteritidis by the rat fleas Xenopsylla cheopsis and Nosophsyllus fasciatus. Pub. Health Reps. 64:933-941.

Galton, M. M., G. K. Morris, and W. T. Martin. 1968. Salmonellae in foods and feeds--Review of isolation methods and recommended procedures. Nat. Commun. Dis. Center. Atlanta, Ga. 41 pp.

Galton, M. M., J. H. Steele, and K. W. Newell. 1964. Epidemiology of salmonellosis in the United States. In The world problem of salmonellosis. E. Van Oye, Ed. Dr. Junk Publishers--The Hague, Brussels. 606 pp.

Greenberg, B. 1964. Experimental transmission of Salmonella typhimurium by houseflies to man. Amer. J. Hyg. 80:149-156.

Greenberg, B., and A. A. Bornstein. 1964. Fly dispersion from a rural Mexican slaughterhouse. Amer. J. Trop. Med. Hyg. 13:881-886.

Greenberg, B., G.Varela, A. Bornstein, and H. Hernandez. 1963. Salmonellae from flies in a Mexican slaughterhouse. Amer. J. Hyg. 77:177-183.

Griffiths, D. A., A. C. Hodson, and C. M. Christensen. 1959. Grain storage fungi associated with mites. J. Econ. Entomol. 52:514-518.

Harein, P. K., and E. De las Casas. 1968. Bacteria from granary weevils collected from laboratory colonies and field infestations. J. Econ. Entomol. 61:1719-1720.

Harein, P. K., E. De las Casas, B. S. Pomeroy, and M. D. York. 1970. Salmonella spp. and serotypes of Escherichia coli isolated from the lesser mealworm collected in poultry brooder houses. J. Econ. Entomol. 63:80-82.

Howe, R. W. 1951. The movement of grain weevils through grain. Bull. Entomol. Res. 42:125-134.

Husted, S. R., R. B. Mills, V. D. Foltz, and M. H. Crumrine. 1969. Transmission of Salmonella montevideo from contaminated to clean wheat by the rice weevil. J. Econ. Entomol. 62:1489-1491.

Julseth, R. M., J. K. Felix, W. E. Burkholder, and R. H. Deibel. 1969. Experimental transmission of Enterobacteriaceae by insects. I. Fate of Salmonella fed to the hide beetle Dermestes maculatus and a novel method for mounting insects. Appl. Microbiol. 17:710-713.

Liu, P. Y., S. H. Zia, and H. L. Chung. 1937. Salmonella enteritidis infection by parenteral route. Proc. Soc. Exp. Biol. Med. 37:17-18.

Mackerras I. M., and M. J. Mackerras. 1949. An epidemic of infantile gastroenteritis in Queensland caused by Salmonellae bovis-morbificans (Basenau). J. Hyg. 47:166-181.

Misra, C. P., C. M. Christensen, and A. C. Hodson. 1961. Angoumois grain moth, Sitotroga Cerealella, and storage fungi. J. Econ. Entomol. 54:1032-1033.

Olson, T. A., and M. E. Rueger. 1950. Experimental transmission of Salmonella oranienburg through cockroaches. Pub. Health Reps. 65:531-540.

Ostrolenk, M., and H. Welch. 1942. The housefly as a vector of food poisoning organisms in food producing establishments. Amer. J. Pub. Health. 32:487-494.

Parker, R. R., and E. A. Steinhaus. 1943. Salmonella enteritidis: Experimental transmission by the rocky mountain wood tick Dermacentor andersoni Stiles. Pub. Health Reps. 58:1010-1012.

Sikorowski, P. P. 1964. Interrelation of fungi and insects to deterioration of stored grains. Wash. State Ag. Exp. Sta. Tech. Bull. 42, 35 pp.

Thomas, S. B., and P. M. Hobson. 1955. Coli-aerogenes bacteria isolated from ears and panicles of cereal crops. J. Appl. Bact. 18:1-8.

U.S. Department of Health, Education, and Welfare. 1964. Salmonella surveillance, annual summary, 1963. Nat. Commun. Dis. Center Publ., Atlanta, Ga.

Van Wyk, J. H., A. C. Hodson, and C. M. Christensen. 1959. Microflora associated with the confused flour beetle, Tribolium confusum. Ann. Entomol. Soc. Amer. 52:452-463.

Welch, H., M. Ostrolenk, and M. T. Bartram. 1941. Role of rats in the spread of food-poisoning bacteria of the Salmonella group. Amer. J. Pub. Health. 31:332-340.

DISSEMINATION OF *SALMONELLA MONTEVIDEO* IN MASSES OF WHEAT BY THE RICE WEEVIL, *SITOPHILUS ORYZAE* (L.)

by

DAVID JAMES SCHUSTER

B. S., Kansas State University, 1970

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

The capability of the rice weevil, Sitophilus oryzae (L.), to disseminate Salmonella montevideo through wheat masses was studied. Data were collected on how far and in what numbers the organism was transferred.

Preliminary tests to compare three wheat sampling techniques were conducted by introducing contaminated insects into the center of small wheat masses in metal drums 21.6 cm diam by 24.2 cm deep. Sampling cages, that were made of 3 mm hail screen and filled with wheat, were considered the most convenient and consistent sampling means.

Tests using a larger grain mass in a metal drum 40 cm diam by 52.5 cm deep with contaminated insects in the center showed that the recovery of S. montevideo on wheat was greatest where the weevils were most numerous. Every sampling area in the drum was contaminated in at least one replicate while samples from an insect-free control were all negative.

One 1.5 m long by 6.25 cm by 6.25 cm wooden trough filled with wheat was infested at one end with weevils exposed to wheat contaminated with 10^4 S. montevideo/g and another trough with weevils exposed to 10^7 S. montevideo/g. Insects were positive in the latter trough as far as 55 cm from the point of introduction. In troughs the same as above with weevils previously exposed to wheat contaminated with 10^7 S. montevideo/g the weevils were able to transfer the organism the full length of the trough in two of three replicates. An insect-free control produced all negative samples.

A cylindrical metal chamber 311.25 cm long and 7.5 cm diam was filled with wheat and infested at one end with weevils previously exposed to wheat contaminated with 10^7 S. montevideo/g. Positive wheat samples were recovered as far away from the infestation point as 270 cm. Salmonella montevideo was present in low numbers ranging from .92 to >1.60/g at 30 cm from the infestation point to .000 to .004/g at 270 cm.

ACKNOWLEDGMENTS

Special appreciation is expressed to Dr. Robert Mills, major professor, who offered valuable advice and aid.

The late Professor V. D. Foltz of the Division of Biology gave counsel during the early directions of this study.

Mr. Martin Crumrine, Graduate Research Assistant in Bacteriology, gave ideas and assistance in formulating methods employed. He also assisted in contaminating the wheat and isolating Salmonella montevideo from wheat and insect samples.

Appreciation is also expressed to Professor John O. Harris of the Division of Biology and Dr. Gerald Wilde, Assistant Professor of Entomology, for serving on the supervisory committee.

The United States Department of Agriculture, Agriculture Research Servic, Market Quality Research Division, Stored Products Insects Research Branch, provided partial support for this study under Cooperative Agreement No. 12-14-100-9403(51) to the Food and Feed Grains Institute (Dr. William Hoover, Director) entitled "The Role of Stored-Product Insects in Distributing Potentially Hazardous Bacteria in Stored Grains".