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Transmission of *Salmonella montevideo* in Wheat by Stored-Product Insects[1]

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Sitophilus oryzae (L.), *S. granarius* (L.), *Tribolium castaneum* (Hbst.), *Oryzaephilus surinamensis* (L.), *Rhyzopertha dominica* (F.), *Tenebroides mauritanicus* (L.), and *Cryptolestes pusillus* (Schon.) transmitted *Salmonella montevideo* from wheat contaminated with 10^6 organisms/g to clean wheat. The insects were fed on the contaminated grain for 21 days and were then transferred to clean grain and allowed to feed for 21 days. They were subsequently transferred to two more samples of clean wheat. All species carried *S. montevideo* into the initial sample of clean wheat but not into a second or third sample. Progeny of the original insects that developed in the contaminated wheat exhibited less ability than the original adults to contaminate clean wheat. Data indicated that few *S. montevideo* could be carried by the stored-product insects in large masses of grain.

Recently, grain and grain products have been implicated as sources of *Salmonella* in food products and animal feeds (1, 14, 15). Human infections occurred in Sweden when *Salmonella*-contaminated barley products were consumed by infants (13).

Several types of insects have been reported to carry *Salmonella* (4-9, 11, 12). Stored-product insects infesting *Salmonella*-contaminated wheat might become carriers of *Salmonella* and, subsequently, contaminate *Salmonella*-free grain.

To study that possibility, we used *S. montevideo* because it has been reported to be one of the 10 most frequent isolates from human and non-human sources in the United States.

MATERIALS AND METHODS

Insects. The insects used, from the Midwest Grain Insects Investigations Laboratory, Market Quality Division of the Agricultural Research Service, U.S. Department of Agriculture, Manhattan, Kan., included the following species: *Sitophilus granarius* (L.), the granary weevil; *S. oryzae* (L.), the rice weevil; *Oryzaephilus surinamensis* (L.), the saw-toothed flour beetle; *Tribolium castaneum* (Hbst.), the red flour beetle; *Rhyzopertha dominica* (J. du V.), the lesser grain borer; *Tenebroides mauritanicus* (L.), the cadelle; and *Cryptolestes pusillus* (Schon.), the flat grain beetle. All are common pests of grain throughout the United States. Both the insects and the media used in rearing colonies were periodically checked for *Salmonella*. No *Salmonella* were isolated from the insects or the rearing media.

Wheat. Wheat used was also obtained from the Midwest Grain Insects Investigations Laboratory. It was cleaned and stored under normal conditions without pesticide treatment. Each lot of wheat was cultured for *Salmonella* before use. No *Salmonella* cells were isolated from the wheat.

Preliminary study. The initial experiment was to determine whether selected insects placed in an *S. montevideo*-contaminated environment could become carriers of *S. montevideo* and whether their progeny, raised in a contaminated environment, could also become carriers. Insects used were the rice weevil, the lesser grain borer, and the red flour beetle. Separate cultures of the three were established in 0.5-gal jars. Each jar contained 100 1-week-old insects and 500 g of wheat contaminated with 10^6 *S. montevideo* per g. The jars were stored at 27 C in a Fisher incubator (model B2), with no attempt to control relative humidity. After 21 days at 27 C, the insects were removed from the wheat by screening on 10 mesh/inch screens. A second screening, using 40 mesh/inch screen, was done to remove loose dust and wheat particles from the insects. The wheat was then returned to the incubator to allow eggs laid by the adult insects to develop into adults. The second generation insects were removed from the wheat approximately 21 days after they emerged as adults. They were cultured for *S. montevideo* as were the original insects.

After the insects were removed from the wheat, they were placed in 10 ml of Brilliant Green tetrathionate (BGTet; Difco) and crushed with a sterile glass rod. The BGTet broth tubes were incubated at 37 C. After 24 and 48 hr in Brilliant Green agar

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(BGA; Difco), plates were streaked from each tube and incubated for 24 hr at 37 C. Typical colonies from each BGA plate were inoculated into triple sugar iron (TSI; Difco) slants, which were incubated for 24 hr at 37 C. Typical *Salmonella* cultures on TSI slants were reacted with *Salmonella* poly H and poly O antisera (Difco). Cultures agglutinating in both antisera were further tested against *Salmonella* O antiserum factor 7 (Difco). *S. montevideo* is an O group C_1 organism, and factor 7 is the identifying antigen for this O group. Thus, any isolated culture that agglutinated in all three antisera was assumed to be *S. montevideo*.

When the second group of insects was removed from the wheat, one 100-g sample of wheat was removed from each culture jar and cultured for *S. montevideo*. Each 100-g sample was placed in 330 ml of BGTet broth and incubated at 37 C. Isolation and identification of *S. montevideo* from the wheat samples was accomplished following the same procedure as described for the insects.

Expanded study. In the next series of tests, we examined the ability of seven species of stored-product insects to carry *S. montevideo* from one sample of wheat to another. The insects used were the rice weevil, the granary weevil, the red flour beetle, the lesser grain borer, the cadelle, the saw-toothed grain beetle, and the flat grain beetle. Separate cultures of the seven species were established in 0.5-gal jars, each containing 500 g of wheat contaminated with 10^6 *S. montevideo* per g. The cadelle culture contained 250 1-week-old adult insects; cultures of the other 6 species contained 500 1-week-old adult insects. The jars were kept at 27 C for 21 days. After 7, 14, and 21 days of storage, 10 insects were removed from each culture and examined for *S. montevideo*, following previously discussed isolation and identification procedures. After 21 days, each culture of insects was screened from the contaminated wheat and placed in 500 g of clean wheat (set 1). The contaminated wheat samples were returned to the incubator and held until progeny insects emerged.

After 21 days in clean wheat, each culture of insects was removed and placed in a second 500 g of clean wheat (set 2) for 21 days. The 500-g wheat samples from each insect culture in set 1 were divided into five 100-g samples and examined for *S. montevideo* as previously discussed. After 21 days in set 2, the insects were removed from each sample and placed in a third group of 500-g wheat samples (set 3). The wheat samples of set 2 were then examined for *S. montevideo* as previously described.

RESULTS AND DISCUSSION

The initial insect study was used as a base for the expanded study. After 21 days in an *S. montevideo*-contaminated environment, each of the three species of insects was carrying *S. montevideo*. Progeny of the insects reared in the same wheat samples were also carrying *S. montevideo*. Progeny of the original adult insects were removed approximately 50 days after the wheat was contaminated with *S. montevideo*. This suggests that insects may become contaminated while in grain previously contaminated.

Each of the seven species used in the expanded study was carrying *S. montevideo* after 7 days in contaminated wheat. Each of the seven species also carried *S. montevideo* into clean wheat (set 1). *S. montevideo* was not carried into a second sample of clean wheat by any of the insects studied. The insects were removed from the wheat in set 3 and cultured for *S. montevideo* 7 days after the transfer from set 2, when the wheat samples of set 2 were found to be free from *S. montevideo*. Each of the seven species of insects was still carrying *S. montevideo*.

The data show that the seven species of stored-product insects studied could transmit *S. montevideo* from contaminated wheat to clean wheat. Transmission of *S. montevideo* from the grain in set 1 to that in set 2 was not observed, although the insects were still carrying *S. montevideo*. Such findings indicate that the number of the test organisms carried by the insects may have been quite low. Some of the *S. montevideo* would not survive exposure to atmospheric conditions in the incubator (3). Although the insects studied could carry *S. montevideo* from one sample of wheat to another, it appeared that they could not transmit large numbers of the test organisms throughout large masses of grain.

Progeny of the insects that developed in the contaminated wheat samples exhibited reduced ability to transmit *S. montevideo* from contaminated wheat to clean wheat. Only progeny of the rice weevil, the saw-toothed grain beetle, and the red flour beetle transmitted *S. montevideo* to clean grain. None of the progeny of those three species transmitted *S. montevideo* to a second sample of clean grain. The insects removed from the first samples of clean wheat, set 1a, carried *S. montevideo*, but none of the insects removed from the second samples of clean wheat, set 2a, carried *S. montevideo*. Such findings further indicate that the seven insect species carried low numbers of *S. montevideo* and that, after being removed from contaminated environment, their ability to transmit *S. montevideo* from one wheat sample to another was limited.

As the number of *Salmonella* in naturally contaminated grain samples is not known, it would be difficult to assess the role of stored-product insects in transmitting *Salmonella* from contaminated grain to clean grain. Under experimental conditions described here, stored-

product insects transmitted low numbers of *S. montevideo* from contaminated wheat to clean wheat, but the role of insects in nature probably is insignificant.

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