Ability of Older *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) Larvae to Infest Whole Corn and Long-Term Population Growth on Whole Corn[1, 2]

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ABSTRACT The rusty grain beetle is a cosmopolitan pest of stored products, and may cause grain to spoil and heat. The ability of 2-week-old rusty grain beetle larvae, *Cryptolestes ferrugineus* (Stephens), to complete development to the adult stage on undamaged corn kernels was compared with development on damaged kernels (undamaged kernels that are slit through the germ with a razor blade) and coarsely cracked corn at 30°C and 75% RH. Both the condition of the corn and infestation level (1 or 5 larvae per cage) significantly affected the proportion of samples in which adults developed. More adults developed in samples containing damaged or coarsely cracked corn than in samples containing undamaged corn. More adults were produced in samples at the higher infestation level. The results indicate that older rusty grain beetle larvae can complete development on sound grain, but more complete development on damaged grain.

A long-term test of the ability of rusty grain beetles to infest whole corn was also conducted. Ten adults and ten 2-week-old larvae were placed on 150 g each of: 1) undamaged kernels, 2) damaged kernels, 3) kernels that were sifted to remove dockage but not otherwise selected, and 4) coarsely cracked corn. Population growth at 30°C and 75% RH was monitored over a 36-week period. Population growth on coarsely cracked corn was rapid. This treatment was terminated after 20 weeks, because the corn was deteriorating and population growth was beginning to decline. Final population levels on damaged and unselected corn kernels were high. There was little population growth on undamaged kernels. The results indicate that sound grain is relatively resistant to infestation by rusty grain beetles, even when older larvae are present. Although commercial grain is susceptible to infestation, rate of rusty grain beetle population growth is slow compared to that on damaged grain.

KEY WORDS Insecta, *Cryptolestes ferrugineus,* rusty grain beetle, population growth, larval survival, corn.

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), is a cosmopolitan pest of stored products, particularly of stored grains (Throne 1987). The results of a number of studies indicate that sound grain is moderately resistant to infestation (Rilett 1949, Tuff and Telford 1964, Mathlein 1971, Throne and

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[2] Names of products are included for the benefit of the reader and do not imply endorsement or preferential treatment by USDA.

Culik 1989). Data presented by Throne and Culik (1989) suggest that even whole grain with all seed coats damaged is moderately resistant to infestation.

Previously-mentioned studies were started with eggs or ovipositing females. Thus, the ability of first-instar larvae to infest and the ability of rusty grain beetle adults to oviposit on whole grain were determined. None of the previous studies determined the ability of older larvae to infest whole grain. Sound kernels may be exposed to infestation by older larvae that develop on damaged kernels. Larvae may leave a kernel on which they have fed because they have eaten the most favorable portions, or to search for more favorable food (Rilett 1949). Therefore, the first objective of this study was to determine whether sound and whole, damaged corn kernels are susceptible to infestation by larvae older than first instars used in previous studies.

Continual attack by numerous insects over a long period of time may allow rusty grain beetles to infest otherwise nonsusceptible grain, perhaps through increased searching for suitable feeding sites or through modification of the environment (i.e., increased temperature and moisture content as a result of insect metabolism). The second objective of this study was to determine the ability of adults and older larvae to infest sound grain and cleaned grain (indicative of commercially-stored grain) during a period of time that would allow for development of several generations of the insect.

Materials and Methods

Insects were taken from cultures reared on medium cracked corn (corn that passed a U. S. standard number 6 sieve with 3.35-mm openings but was retained on U. S. standard number 20 sieve with 0.85-mm openings) at 30°C, 60 to 65% RH, and a 12-h photoperiod. Cultures were started with rusty grain beetle adults obtained in or around bins of corn on farms in Bamberg and Barnwell counties, South Carolina.

The corn (*Zea mays* L.) used was 'Pioneer 3320,' the cultivar most commonly grown in the southeastern United States. It was grown in 1987 in South Carolina. Corn was screened over a number 6 sieve to remove dockage (small particles of grain and foreign material), and frozen at ca. -1°C for at least two weeks to kill insects that may have been present.

Ability of Larvae to Infest Undamaged Kernels. The ability of older larvae to infest undamaged corn kernels was determined by placing either one or five 2-week-old larvae in cages containing undamaged kernels (kernels with no breaks in the seed coat visible to the naked eye). Additional treatments were: 1) damaged kernels (whole kernels slit < 1 mm deep longitudinally through the germ with a razor blade), and 2) coarsely cracked corn (corn cracked in a blender to pass a sieve with 6.3-mm openings but retained on a number 6 sieve). Thus, there were six treatments - two infestation levels by three corn treatments.

Insects were tested in cages made by joining the uncovered ends of two cylindrical plastic tubes (26 mm O. D. by 19 mm I. D. by 13 mm H), each covered at one end with a 64-μ-mesh nylon screen, and securing the tubes with a rubber band. Five kernel equivalents (1.6 ± 0.1 g) of corn were placed in each of ten cages for each treatment.

All phases of the experiment were conducted at 30° ± 1°C and a predicted relative humidity of 75% RH, conditions that are close to optimal for rusty grain beetle development (Smith 1965). The photoperiod was 12 h daily. The corn was brought to the required humidity by placing the cages containing the treatments randomly on a false floor in a plastic box (40 by 27.5 by 16 cm H) containing a saturated sodium chloride (NaCl) solution (Greenspan 1977). A cage (base: 86 mm O. D. by 84 mm I. D. by 80 mm H; lid: 89 mm O. D. by 88 mm I. D. by 8 mm H) containing 300 g of whole, but not necessarily undamaged, corn was placed in the plastic box to check the moisture content of the corn weekly using a Motomco® model 919® automatic grain moisture tester (Dickey-John Corp., Auburn, IL). The corn was equilibrated for 6 weeks.

To obtain larvae of known age, four weeks into the equilibration period 400 3-4-week-old third- or fourth-laboratory-generation rusty grain beetle adults were placed on ca. 0.25 liter of medium cracked corn in 0.473-liter jars with 100 adults per jar. Adults were allowed to oviposit for 24 h, and then were removed. The jars were covered with a canning jar lid that was modified by drilling a 3.3-cm hole in the lid and soldering a brass screen with 0.425-mm openings over the hole. Filter paper was placed on both sides of the lid.

At the end of the equilibration period, either one or five 2-week-old larvae were placed in each of ten cages of each corn treatment. Larvae reared for two weeks at 30°C and 75% RH should be third instars (Bishop 1959). Starting three weeks after larvae were placed in the cages, the grain was visually examined for adults by pouring into a small dish. Adults were counted and removed. The grain was checked weekly for adult emergence until all larvae had completed development or died.

The experiment was replicated three times. Within each replication, cages were arranged in a completely randomized design. The response used in analyses was the proportion of the ten cages for each treatment within a replication in which an adult developed. The third replication of five larvae on undamaged kernels was based on data for only nine cages, because the larvae escaped through a hole in the screen in one of the cages. Data were analyzed using the general linear models procedure (GLM; SAS Institute 1987).

Long-Term Population Growth. Lots of 150 ± 0.1 g each of undamaged kernels, damaged kernels, coarsely cracked corn, and unselected kernels (sieved over a number 6 sieve to remove dockage) were placed into each of three 0.5-pint (0.237 liter) canning jars and covered with a modified canning jar lid/filter paper combination. The initial moisture content of the corn was 14.6%. Ten 4- to 11-day-old, third-laboratory-generation adults and ten 0- to 13-day-old, fourth-laboratory-generation larvae were placed in each jar. The jars were randomly placed on a false floor in a plastic box (40 by 27.5 by 16 cm H) containing a saturated sodium chloride solution. An additional two jars containing 150 ± 0.1 g each of whole, but not necessarily undamaged, corn were placed in the plastic box to check the moisture content of the corn weekly using a Motomco® model 919® automatic grain moisture tester. The plastic box was placed in an incubator maintained at 30° ± 1°C and with a 12-h photoperiod. Adults were sieved from the corn every four weeks for 36 weeks, counted, and placed back into the jars. After 36 weeks, all kernels that showed signs of feeding damage were cut open with a razor blade to check for adults that might be in the kernels.

Jars were arranged in a completely randomized design. Data were analyzed using analysis of variance (ANOVA; SAS Institute 1987).

Results and Discussion

Ability of Larvae to Infest Undamaged Kernels. Moisture content of the 300-g samples of corn ranged from 14.2-14.9%. The condition of the corn [F = 135.8; df = 2, 12; P (F > 135.8) = 0.00] and the number of larvae in a cage [F = 6.1; df = 1, 12; P (F > 6.1) = 0.03] both affected the proportion of samples in which adults developed (Table 1). The corn condition by number of larvae in a cage interaction was not significant [F = 0.8; df = 2, 12; P (F > 0.8) = 0.46].

More samples had adults develop when there were five larvae per cage than when there was one larva per cage. Linear contrasts showed that more samples had adults develop when the grain was damaged (slit or cracked) than when it was undamaged [F = 271.2; df = 1, 12; P (F > 271.2) = 0.00]; whether the grain was cracked or slit did not affect the number of samples that had adults develop [F = 0.44; df = 1, 12; P (F > 0.44) = 0.52].

Table 1. Proportion ($\overline{X}$ ± SD) of cages in which rusty grain beetle larvae survived to the adult stage on corn of varying condition.*

	No. larvae per cage	
Corn condition	1	5
Undamaged	0.20 ± 0.00	0.24 ± 0.11
Damaged	0.83 ± 0.15	1.00 ± 0.00
Coarsely cracked	0.90 ± 0.10	1.00 ± 0.00

*Number of observations (n) per treatment is 3.

Rusty grain beetles normally enter a grain bin as adults. These colonizing adults lay eggs in the grain and resulting larvae feed on grain, particularly damaged particles. Larvae feeding on particles that could not support development to the adult stage would eventually leave those particles to look for more favorable food (Rilett 1949). Thus, grain would be exposed to infestation by older larvae. The results of the present study indicate that older larvae readily infested damaged corn kernels. A few larvae were able to survive to the adult stage on undamaged kernels, perhaps because these kernels were not examined microscopically for breaks in the seed coat. There may have been one or a few sites in each cage where the larvae could burrow into the kernels. Or, the larvae may have pupated and emerged without feeding. However, survival on sound kernels was reduced compared to that on damaged kernels. Whole, damaged kernels were as susceptible to infestation by older rusty grain beetle larvae as was coarsely cracked corn. Previous studies had indicated that fewer progeny were produced on whole, damaged kernels than on coarsely cracked corn (Throne and Culik 1989, Throne 1990), perhaps because fewer eggs are laid on whole kernels (Throne 1991). Apparently, older larvae are capable of infesting or completing development on whole, damaged kernels.

Long-Term Population Growth. Moisture content of the 300-g sample of corn used to monitor moisture content ranged from 14.1 - 14.8%. The population on coarsely cracked corn grew to an average of 725 (SEM = 14.47, n = 3) live adults per jar after 20 weeks. This treatment then was terminated because about one-third of the contents of the jars was reduced to dust and frass, and rate of population growth had declined. The data for coarsely cracked corn are not presented in Table 2, because these data were collected for 20 weeks: data for the other treatments were collected for 36 weeks.

In the other treatments with uncracked corn, about as many rusty grain beetles were inside the kernels at the final count as were sieved from the corn. Therefore, data from the censuses taken every four weeks are not presented. Only data from counts taken after kernels were cut open were used in the analyses.

The number of live rusty grain beetles in the samples at the end of the test (Table 2) varied significantly with corn condition [$F = 213.0$; df = 2, 6; $P (F > 213.0) = 0.00$]. The total number of adults produced during the study (defined as live adults at 36 weeks plus the sum of the dead adults removed every four weeks minus the original 10 adults) was transformed using the Box-Cox transformation (Box and Cox, 1964) before analysis to stabilize the variances. However, transformation did not affect the results, so all statistics reported are for untransformed data. The total number of adults produced also varied significantly with corn condition [$F = 229.5$; df = 2, 6; $P (F > 229.5) = 0.00$]. More adult progeny were produced on damaged kernels than on the unselected corn kernels, which in turn yielded more progeny than undamaged kernels.

Table 2. Number ($\bar{X} \pm$ SEM) of live rusty grain beetle adults after 36 weeks and total number of adult progeny produced in 36 weeks in corn of varying conditions.

	Number of rusty grain beetle adults*	
Corn condition	Live	Total†
Damaged	493.3 ± 25.2 a	860.3 ± 48.4 a
Unselected	120.0 ± 14.5 b	160.7 ± 15.2 b
Undamaged	22.7 ± 5.0 c	29.3 ± 6.1 c

* Number of observations (n) per treatment is 3. Means within a column that are followed by different letters are significantly different ($\alpha = 0.05$; Waller-Duncan k-ratio test).

† Defined as live adults at 36 weeks plus the sum of the dead adults removed every four weeks minus the original 10 adults.

It is obvious from Table 2 that damaged kernels are vulnerable to rusty grain beetle attack. Most of the kernels that were damaged by slitting with a razor blade had rusty grain beetle adults trapped or sheltered inside. Throne and Culik (1989) and Throne (1990) reported that few rusty grain beetle adult progeny were produced on kernels that had been damaged by slitting with a razor blade, and Throne (1991) reported that few eggs are laid on slit kernels. In the present study, as many as five generations of rusty grain beetles may have developed (Throne and Culik 1989). In early generations, few progeny may have been produced in a small number of kernels. However, once damaged, these kernels may have become more suitable for rusty grain beetle development and more rapid population growth. Supporting this hypothesis is the fact that when cutting open damaged corn kernels after the 36-week sieving, I often found six to ten adults in a hollowed out kernel. Although these damaged kernels were susceptible to rusty grain beetle attack, they were not as favorable for population growth as the cracked corn. Almost as many adult progeny were produced on cracked corn as on the damaged kernels, but in half the time.

About one-fifth as many adults were produced on the unselected corn kernels as on the damaged kernels. Population growth on the unselected corn kernels is indicative of productivity in an actual storage situation, because this treatment contained kernels that were in all conditions: broken, cracked, and undamaged, but no dockage. Approximately 70 - 80% of the kernels were undamaged, which probably explains why one-fifth as many adult progeny were produced as on the damaged kernel treatment.

About three percent as many adults were produced on undamaged kernels as on the damaged kernels. Only about 30 adults were produced per jar over a period of time when approximately five generations were completed on damaged kernels. These results corroborate those from short-term studies (Throne and Culik 1989, Throne 1990), indicating that rusty grain beetle population growth is slow on undamaged corn kernels. Adding older larvae to the sound kernels did not enhance population growth on sound corn.

The conditions in this study were very unfavorable for storing grain. High temperature and high moisture content were chosen for optimal rusty grain beetle growth. Also, the infestation rate, comparable to about 1,690 adult and 1,690 larval rusty grain beetles per bushel, was much higher than the number of grain beetles required for grain to be considered infested, 254 live beetles per bushel (USDA, FGIS 1988). Storage at lower temperatures and humidity is recommended and attainable, even in the southeastern U. S. by judicious use of aeration fans. The length of this study might be comparable to two to three years in actual storage in the southeastern U. S. and comparable to even longer storage periods in colder climates. Based on the results of the present study, corn stored below 15% moisture content with an intact seed coat should be free from significant rusty grain beetle damage during long-term storage. Even corn with 10 to 20% damaged kernels probably could be stored safely for a few years, if initial infestation levels are low. Field validation of these results is necessary.

This study has shown for the first time that older rusty grain beetle larvae can complete development on sound grain, although at a lower rate than on damaged kernels. Also, for the first time, the study has shown that despite the presence of a mixed-age population of rusty grain beetles and poor environmental conditions for storage, population growth of rusty grain beetles on sound and cleaned grain is slow.

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