Intra- and interspecific interactions among *Tribolium castaneum* and *Cryptolestes ferrugineus* in stored wheat at different insect densities

[Interactions intra et interspécifiques de *Tribolium castaneum* et *Cryptolestes ferrugineus* à diverses densités dans le blé entreposé]

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Résumé de l'article

Cryptolestes ferrugineus et *Tribolium castaneum* sont des ravageurs des grains entreposés responsables d'importantes pertes économiques au Canada. Ces insectes ont été élevés en laboratoire (30°C et 70 % HR) séparément ou ensemble sur du blé roux vitreux de printemps à trois densités d'insectes, soit 1000, 500 et 250 adultes par kilogramme de blé pour les espèces séparées, et au double de ces densités pour les espèces mélangées. Cette expérience a été menée avec du blé sec (12 % de contenu en humidité [CH] ou de teneur en eau) et humide (15 % de CH ou de teneur en eau). Les adultes ont été échantillonnés aux 2 semaines sur une période de 10 semaines. Le blé tamisé a été incubé à 30°C et 70 % HR durant 4 semaines afin d'observer le nombre d'adultes qui ont émergé des insectes immatures présents sous les téguments des germes de blé. Toutes les expériences ont été répétées quatre fois. La densité initiale des insectes, le contenu en humidité et la combinaison des espèces ont eu des impacts significatifs sur la population finale. Le nombre d'adultes des deux espèces a été plus élevé avec le blé à 15 % de CH. Avec le blé à 12 % de CH, le nombre de *T. castaneum* a été plus élevé que le nombre de *C. ferrugineus* lorsqu'ils ont été élevés séparément, alors que ce fut le contraire avec le grain à 15 % de CH. Les populations de *Cryptolestes ferrugineus* ont été significativement plus élevées lorsque élevées avec le *T. castaneum* que lorsque élevées isolément sur le grain à 12 % de CH. Cette tendance a été inversée avec le grain à 15 % de CH. Pour les deux contenus en humidité des grains, le nombre de *T. castaneum* a été plus grand lorsque élevé seul par rapport à un élevage avec le *C. ferrugineus*. L'étude laisse supposer que les populations de *T. castaneum* sont inhibées par la présence de *C. ferrugineus*. Des densités d'insectes élevées accentuent souvent cet effet.

Intra- and interspecific interactions among *Tribolium castaneum* and *Cryptolestes ferrugineus* in stored wheat at different insect densities

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Cryptolestes ferrugineus and *Tribolium castaneum* are important economical pests of stored grain in Canada. Insects were reared separately or together on hard red spring wheat at three insect densities representing 1000, 500, and 250 adults per kilogram of wheat for single species and twice that of single densities for mixed species, in the laboratory (30°C, 70% RH). The experiment was conducted on dry (12% moisture content [MC], wet basis) and damp wheat (15% MC, wet basis). Adults were sampled every 2 weeks over a 10-week period. Sifted wheat was incubated at 30°C, 70% RH for 4 weeks to observe the number of adults emerging from immatures present under the seed coat of the wheat germ. All experiments were replicated four times. Initial insect densities, moisture content, and species combination had significant effects on the resulting adult population. Adult numbers for both species were higher on 15% MC wheat. In 12% MC wheat, *T. castaneum* numbers were higher than *C. ferrugineus* numbers when reared separately whereas this was reversed in 15% MC grain. *Cryptolestes ferrugineus* populations were significantly higher when reared with *T. castaneum* than when reared as a single species on 12% MC grain. The trend was reversed in 15% MC grain. At both grain moistures, *T. castaneum* numbers were higher as an isolated species compared to when reared with *C. ferrugineus*. The study suggested that *T. castaneum* populations were inhibited in the presence of *C. ferrugineus*. High insect density often accentuated these effects.

[Interactions intra et interspécifiques de *Tribolium castaneum* et *Cryptolestes ferrugineus* à diverses densités dans le blé entreposé]

Cryptolestes ferrugineus et *Tribolium castaneum* sont des ravageurs des grains entreposés responsables d'importantes pertes économiques au Canada. Ces insectes ont été élevés en laboratoire (30°C et 70 % HR) séparément ou ensemble sur du blé roux vitreux de printemps à trois densités d'insectes, soit 1000, 500 et 250 adultes par kilogramme de blé pour les espèces séparées, et au double de ces densités pour les espèces mélangées. Cette expérience a été menée avec du blé sec (12 % de contenu en humidité [CH] ou de teneur en eau) et humide (15 % de CH ou de teneur

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en eau). Les adultes ont été échantillonnés aux 2 semaines sur une période de 10 semaines. Le blé tamisé a été incubé à 30°C et 70 % HR durant 4 semaines afin d'observer le nombre d'adultes qui ont émergé des insectes immatures présents sous les téguments des germes de blé. Toutes les expériences ont été répétées quatre fois. La densité initiale des insectes, le contenu en humidité et la combinaison des espèces ont eu des impacts significatifs sur la population finale. Le nombre d'adultes des deux espèces a été plus élevé avec le blé à 15 % de CH. Avec le blé à 12 % de CH, le nombre de *T. castaneum* a été plus élevé que le nombre de *C. ferrugineus* lorsqu'ils ont été élevés séparément, alors que ce fut le contraire avec le grain à 15 % de CH. Les populations de *Cryptolestes ferrugineus* ont été significativement plus élevées lorsque élevées avec le *T. castaneum* que lorsque élevées isolément sur le grain à 12 % de CH. Cette tendance a été inversée avec le grain à 15% de CH. Pour les deux contenus en humidité des grains, le nombre de *T. castaneum* a été plus grand lorsque élevé seul par rapport à un élevage avec le *C. ferrugineus*. L'étude laisse supposer que les populations de *T. castaneum* sont inhibées par la présence de *C. ferrugineus*. Des densités d'insectes élevées accentuent souvent cet effet.

INTRODUCTION

Stored grain on farms in Western Canada is often infested by two major granivorous pests: the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) [Coleoptera: Laemophloeidae], and the red flour beetle, *Tribolium castaneum* (Herbst) [Coleoptera: Tenebrionidae] (Madrid *et al.* 1990). These two species are cosmopolitan in distribution but differ in food habits, tolerance for abiotic physical factors, and population characteristics. *Cryptolestes ferrugineus* is cold-hardy, tolerates low relative humidities down to 10% (Howe 1965), can survive to -15°C for several wk after acclimatization (Fields 1992) and its population can increase 60-fold in 4 wk (Sinha and Watters 1985). *Tribolium castaneum* is cold-susceptible, has greater tolerance to low humidities as low as 1% (Howe 1965), thrives on broken kernels, dockage, and flour and populations can increase 70-fold in 4 wk (Sinha and Watters 1985). The range of temperature in which stored-product insects can normally survive lies between 8-41°C (Sinha and Watters 1985), and most economically important insect pests of stored-products develop and reproduce over a narrower optimal range of temperature between 20-35°C (White 1995). Generally, development and multiplication are optimal near 30°C

and 50-70% RH (Howe 1965). *Cryptolestes ferrugineus* successfully develops at 20-40°C and 40-95% RH (optimum 32-35°C and 70-90% RH) and *T. castaneum* at the same temperatures but 10-95% RH (optimum 70-75% RH) (Sinha and Watters 1985).

Canadian grain is usually stored below 20°C except immediately after harvest when grain can be binned at temperatures of 30°C or more (Kawamoto *et al.* 1991). Wheat is stored at a straight grade of 14.5% moisture content (MC) or less (Canadian Grain Commission 2002) which has an equilibrium RH of 70% (Muir 1973). In years with wet harvest conditions, grain can be placed into storage at higher than straight grade MC which can promote mold growth. Grain can also be stored from several mo up to 2 yr in large non-aerated grain bulks (Jayas *et al.* 1991) which remain warm in the bulk centre. These conditions are favorable for development of stored-product pests.

Numerous studies (Hagstrum and Throne 1989; Kabir 1966; Lefkovitch 1968; White and Sinha 1980) have been conducted in the laboratory on interactions among various species of stored-product beetles. Kabir (1966) reported laboratory interactions among *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), and *T. castaneum* on sorghum and

found that *R. dominica* and *S. oryzae* mutually inhibited development of each other whereas *T. castaneum* benefited from the presence of either or both species. Lefkovitch (1968) observed laboratory interactions among *S. oryzae*, *Lasioderma serricone* (F.), *T. castaneum*, and *C. ferrugineus* at 30°C and 60% RH. He reported that on both wheat and wheat-feed, *C. ferrugineus* inhibited *T. castaneum* and that *C. ferrugineus* would be the most likely to survive among all four species (Lefkovitch 1968).

The objective of this research was to study adult populations of *C. ferrugineus* and *T. castaneum* separately and together under insect densities of 250, 500, and 1000 insects per kg in 12% and 15% MC wheat, respectively. These MC represent an equilibrium RH of 60% and 75%, respectively (Muir 1973). The questions addressed were whether: 1) any one species had an inhibitory effect on the population of the other species when they are reared together compared to their numbers in isolation; 2) insect density had a significant effect on adult populations of the two species in isolation or in combination; 3) initial grain moisture had a significant effect on adult populations of the two species. Data from this experiment may be used to develop ecological models which could aid extension personnel or grain storage managers in predicting potential storage problems related to insect infestation.

MATERIALS AND METHODS

Insects and Sex Ratio

Cryptolestes ferrugineus were reared in laboratory cultures on whole wheat plus wheat germ (19:1, wt:wt) and *T. castaneum* were reared on wheat flour plus Brewer's yeast (19:1, wt:wt), at 30 ± 1°C and 70 ± 5% RH. The adults of both species used in the experiments were 2- to 3-wk-old. Sexing of *T. castaneum* was done by identifying the sex of pupae based on the pupal genital papillae in Tenebrionidae (Halstead 1963) and of *C. ferrugineus* as adults based on a mandibular projection on males (Rilett 1949). Three replicates each of 100 pupae of *T. castaneum* and

adults of *C. ferrugineus*, were sexed in the laboratory. The mean sex ratio of 100 individuals was 51:49 for *T. castaneum* and 35:65 for *C. ferrugineus* (males to females). These values for sex ratio for the two species are similar to those reported by other researchers (Kawamoto *et al.* 1989; Rilett 1949; Smith 1966).

Experimental Setup

Certified hard red spring wheat, cultivar AC Barrie, with 5% dockage (finely ground wheat) by weight, and at two moisture contents of 12% and 15% wet basis (wb) was used as food for both insect species. Glass bottles of 224 mL capacity and 10 cm diam with perforated lids were used to store the grain with insects. Each glass bottle contained 150 g of wheat inclusive of 5% dockage. Adult insects of the two species were inserted singly or together at three densities. Hence a glass bottle with 150 g of wheat contained 37, 75, and 150 adults of each species, designated as low, medium, and high insect densities, respectively. For species reared together, each glass bottle contained 37, 75, and 150 adults of each species, for a total of 74, 150, and 300 insects at low, medium, and high insect densities, respectively. Adult counts were taken at 2, 4, 6, 8, and 10 wk. Four replicates were prepared for the three initial insect densities and species combinations (two single species and one in combination), and two moisture contents. Separate sets of bottles were prepared for each time interval. This was done to minimize the error due to repeated handling of the same grain at several time intervals of sampling. In all, 360 bottles were prepared with insects in isolation and combination and 72 bottles were sampled at each of 2, 4, 6, 8, and 10 wk. All bottles were kept in a temperature controlled chamber at 30°C and 70% RH. These standard near-optimal conditions allowed for successful population growth (Sinha and Waters 1985) while preventing fungal contamination (Wicklow 1995).

At the various sampling intervals, the bottles were removed, the grain sieved on a #10 screen (2-mm apertures), and adults carefully removed with an aspi-

ration tube. With the adults removed, the remaining immatures and grain were returned to the bottles and incubated at 30°C and 70% RH for 4 wk to determine the number of adults emerging. No other developmental stage of either species was counted. The number of live adults presented in the data are the adults removed by sieving at the end of each respective sampling interval (2, 4, 6, 8, and 10 wk) plus the number of adults emerging from immatures after 4 wk of incubation.

Statistical analysis

To analyze the effect of various variables, the numbers of adults were cube-root transformed (Steel and Torrie 1960) and analyzed by ANOVA (Proc GLM) (SAS 1995). Insect number was the dependent variable, and insect density, wheat moisture content, and species (separate or combined) were independent variables.

RESULTS AND DISCUSSION

Overall, moisture content and initial insect density had significant effects ($F = 13.65$) on the population of both *T. castaneum* and *C. ferrugineus* regardless of being reared separately or together. Adult populations of both species, irrespective of rearing method, were higher in 15% MC wheat than in 12% MC wheat (Tables 1 and 2). This was expected as humid conditions in damp wheat (above 14.5% MC) are more favourable for multiplication of insects than dry wheat (Howe 1965).

Intraspecific interactions

The singly raised populations of *C. ferrugineus* and *T. castaneum* were significantly affected by moisture content ($F = 9104.46$) and initial insect density ($F = 104.66$) over the span of the experiment. Again, the numbers were lower in 12% MC grain compared to 15% MC grain for both species.

Table 1. Mean $\pm$ SE number of adult *Cryptolestes ferrugineus* reared alone and with *Tribolium castaneum* from three initial insect densities in 150 g of 12% (dry) and 15% (damp) moisture content (MC) wheat at 30°C and 70% relative humidity for all sample dates (2, 4, 6, 8, and 10 weeks)

Initial density (no. adults of each species/150 g)	Reared alone		Reared with <i>T. castaneum</i>	
	12% MC	15% MC	12% MC	15% MC
Low (37)	49 $\pm$ 5	927 $\pm$ 46	125 $\pm$ 22	688 $\pm$ 35
Medium (75)	55 $\pm$ 5	1049 $\pm$ 46	88 $\pm$ 6	760 $\pm$ 56
High (150)	91 $\pm$ 10	1096 $\pm$ 53	133 $\pm$ 6	882 $\pm$ 25

$n = 4$ replicates.

Table 2. Mean $\pm$ SE number of adult *Tribolium castaneum* reared alone and with *Cryptolestes ferrugineus* from three initial insect densities in 150 g of 12% (dry) and 15% (damp) moisture content (MC) wheat at 30°C and 70% relative humidity for all sample dates (2, 4, 6, 8, and 10 weeks)

Initial density (no. adults of each species/150 g)	Reared alone		Reared with <i>C. ferrugineus</i>	
	12% MC	15% MC	12% MC	15% MC
Low (37)	159 $\pm$ 11	397 $\pm$ 29	105 $\pm$ 15	127 $\pm$ 14
Medium (75)	215 $\pm$ 20	483 $\pm$ 26	116 $\pm$ 8	123 $\pm$ 11
High (150)	289 $\pm$ 18	575 $\pm$ 28	179 $\pm$ 8	196 $\pm$ 16

$n = 4$ replicates.

Figures 1 to 4 represent the mean adult population (adult count at each sampling interval plus the adults emerging from immatures after 4 wk of incubation); standard errors (SE) are in parenthesis, for the two species under 12% and 15% MC grain rearing conditions. For *C. ferrugineus* alone, mean populations at all the three insect densities were significantly higher in 15% MC grain than 12% MC grain (Figs. 1 and 2). A similar trend was observed for *T. castaneum* (Figs. 3 and 4). For single species in 12% MC grain, however, mean populations were higher for *T. castaneum* than *C. ferrugineus* over all insect densities (Tables 1 and 2). This observation confirms the slightly higher rate of increase of *T. castaneum* compared to *C. ferrugineus* (Sinha and Watters 1985) and reflects a greater ability of *T. castaneum* to survive over a wider range of relative humidity (White 1995). *Tribolium castaneum* is one of the most cosmopolitan pests of stored cereals and other foods (Sinha and Watters 1985).

For single species in 15% MC grain, overall mean numbers of *C. ferrugineus* at low, medium, and high densities were 2.3-, 2.2-, and 1.9-fold higher, respectively, than *T. castaneum* numbers (Tables 1 and 2). Sinha (1975) observed *C. ferrugineus* adult populations to be 1.2-fold higher than *T. castaneum* in stored wheat with a 5% dockage level after 12 wk. The lower population of *T. castaneum* in 15% MC grain can be attributed to a crowding effect which reduces fecundity (Rich 1956; Sonleitner 1961) and to a high incidence of cannibalism in confined laboratory populations of *Tribolium* (Park 1934). Under extreme circumstances, eggs, pupae, and callow adults have suffered mortality greater than 90% due to predation by larvae and mature adults of their own species (Rich 1956; Sonleitner 1961).

Interspecific interactions

In 12% MC grain, *C. ferrugineus* adult numbers were significantly higher when reared in combination with *T. castaneum* than when reared in isolation, at all

FIGURE 1. Mean $\pm$ SE number of adult *Cryptolestes ferrugineus* at initial low, medium, and high densities, reared alone or in combination with *Tribolium castaneum* in 150 g of 12% MC (dry) wheat. (Numbers are represented by the adult observed at the sampling interval plus the adults eclosing from pupae after incubation of 4 weeks).

FIGURE 2. Mean $\pm$ SE number of adult *Cryptolestes ferrugineus* at initial low, medium, and high densities reared alone or in combination with *Tribolium castaneum* in 150 g of 15% MC (damp) wheat. (Numbers are represented by the adult observed at the sampling interval plus the adults eclosing from pupae after incubation of 4 weeks).

FIGURE 3. Mean $\pm$ SE number of adult *Tribolium castaneum* at initial low, medium, and high densities, reared alone or in combination with *Cryptolestes ferrugineus* in 150 g of 12% MC (dry) wheat. (Numbers are represented by the adult observed at the sampling interval plus the adult eclosing from pupae after incubation of 4 weeks).

FIGURE 4. Mean $\pm$ SE number of adult *Tribolium castaneum* at initial low, medium, and high densities reared alone or in combination with *Cryptolestes ferrugineus* in 150 g of 15% MC (damp) wheat. (Numbers are represented by the adult observed at the sampling interval plus the adults eclosing from pupae after incubation of 4 weeks).

three densities (Fig. 1; Tables 1 and 2). However, this trend was reversed in 15% MC grain, where *C. ferrugineus* populations were significantly higher in isolation than when reared with *T. castaneum* (Fig. 2; Tables 1 and 2). It is possible that in dry grain, *C. ferrugineus* 1st instar larvae were unable to penetrate the wheat germ because of their small size and the intact kernels. Grain damaged by *T. castaneum* feeding was easier for *C. ferrugineus* adults to feed on and for larvae to enter. White (1979), in a preliminary study, also observed that the population of *C. ferrugineus* was higher than *T. castaneum* in wheat at the end of 5, 10, and 15 wk when reared together using an initial 25 adults of each species at 14.5% MC.

Suresh *et al.* (2001) observed that adults of *T. castaneum* caused more mortality of the immatures of both species than *C. ferrugineus* adults. They also observed that *T. castaneum* larvae caused higher mortality to their own species than to *C. ferrugineus*. Our results are consistent with previous

observations, that *C. ferrugineus* has an inhibitory effect on *T. castaneum* populations. At the experimental densities tested, and under closed conditions where insects could not migrate from crowding pressure, cannibalism may have had a severe impact on the populations observed. In most Canadian conditions, where migration is possible, the effects may be tempered as insect densities are less, except for active "hot-spots." The density of *Cryptolestes* spp. found in wheat sampled from farms in northern US states during the summer and fall of 1980 was 45 insects per kg (Storey *et al.* 1983). Populations of *T. castaneum* would be expected to decline slowly because of cannibalism and predation by *C. ferrugineus* adults on the eggs and larvae of *T. castaneum*, while *C. ferrugineus* immatures were largely protected from predation by developing under the seed coat at the wheat germ (Suresh *et al.* 2001). In this study, *T. castaneum* populations remained static, showing little if any increase when combined with *C. ferrugineus*.

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