Monitoring populations of the flour beetles *Tribolium confusum* Jacquelin du Val and *Tribolium castaneum* (Herbst) in flour mills and in laboratory settings

by

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This thesis is dedicated to my late grandmother, Jesse Hawkin, who would have been a brilliant graduate student if she had been allowed the opportunity.

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Abstract

This thesis reports the effects of disturbance and harbourage on the fitness of *Tribolium confusum* and *T. castaneum*, as well as the the efficacy of pheromone monitoring traps for monitoring for populations of *Tribolium* in laboratory and mill settings. Behavioural studies were also carried out on mill and laboratory-reared beetles and the distributions of both species in a mill were examined.

Twenty-four hour sieving disturbance decreased the rates of dispersal for both species, and decreased *T. castaneum* fecundity. Rolling disturbance decreased *T. confusum* dispersal rate while shaking disturbance decreased *T. castaneum* dispersal rate. When undisturbed beetles were given differing amounts of flour in the presence or absence of harbourage, beetles laid more eggs in larger amounts of flour, but harbourage only affected *T. castaneum* at one level of flour (2 g). Throughout disturbance and harbourage experiments, *T. castaneum* laid more eggs than *T. confusum*.

Pheromone monitoring traps placed in three Canadian flour mills were not useful in predicting the degree of infestation inside Simons rollstands. Pheromone monitoring traps also showed low efficacy (i.e. caught few beetles) in both mill and laboratory settings, and *T. confusum* was caught less often than *T. castaneum* in both mills and in a warehouse. Mill-strain beetles of both species were caught less often than laboratory-strain beetles in a warehouse. In one Canadian flour mill, both *T. castaneum* and *T. confusum* were found inside rollstands but the two species were spatially segregated from one another, rarely being found together in the same rollstand. In contrast to this, both species were consistently found together in samples

taken from the same mill less than a year beforehand. In behavioural laboratory studies, beetles collected directly from a mill moved slower than beetles collected from a laboratory culture and this response was shown to be phenotypic. Mill-strain and laboratory-strain beetles also differed in burrowing tendencies, with *T. confusum* from the laboratory strain burrowing less than *T. confusum* from a mill and *T. castaneum* from different mills sometimes burrowing more and sometimes less than *T. castaneum* from the laboratory strain.

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Chapter 1: Introduction

Stored product insects are diverse in terms of their biology and the products they infest. Twenty-six families of insect pests are found in stored products worldwide, in everything from beans and cereals to museum exhibits. The original habitats of these insects include the underside of tree bark, seeds, leaf litter, fruit, fungi and mold, carrion and various nests (Rees, 2004). These insects became cosmopolitan due to increased commerce (Good, 1936) and were spread throughout the world by trade (Rees, 2004).

Commodity damage due to stored product insects is substantial. Stored product insect pests probably cause a 5-10% loss in commodities worldwide, with this number likely to be higher in tropical regions (Mondal and Port, 1994). In many developing countries, damage due to pests like insects and rodents is responsible for much of the overall loss in food quality and quantity because most stored food remains on the farm (Snelson, 1987). In developed countries such as Canada, the presence of insects in and of themselves is damaging. There is zero tolerance for live insects in Canada and tight restrictions are placed on the number of insects fragments that can be present in food (Government of Canada: Health Protection Branch, 1999). Consumers are even more demanding than government, rejecting food with any evidence of insect contamination. For these reasons, control of stored product insects is essential.

There are many chemical and non-chemical methods of insect control available. Chemical methods (fumigants and contact insecticides) are the primary methods of control. These methods are relatively fast-acting, have broad spectrums of activity and most are economical. Methyl bromide is an especially important and common fumigant

used to control insects. However, methyl bromide is an ozone depleter (Ozone Secretariat, 1992) so its use is currently being phased out around the world.

The banning of methyl bromide, along with health and environmental concerns over chemical pest control, has led to the increased use of non-chemical control methods. Non-chemical methods do not have the same negative health and environmental effects as chemical treatments, but they are often narrow in their range of activity, more complicated to use and more expensive than chemical controls. Integrated pest management (IPM) can make non-chemical control methods more accessible and economical, because under IPM one controls for pest populations only when the economic damage of the pests exceeds the cost of control (Subramanyam and Hagstrum, 2000).

Sanitation and pest population sampling are critical to the success of stored product IPM programs. Sanitation directly removes and excludes pests, in addition to disturbing their habitat. There is little known about the effects sanitation can have on the fecundity and dispersal of insect pest populations. Monitoring pest populations through sampling helps determine when pest control is warranted under IPM, but accurate sampling requires knowledge of insect pests, i.e. their distributions, behaviour within and around commodities, interactions with each other and their pheromones.

This thesis is concerned with sanitation and sampling of the red flour beetle, *Tribolium castaneum* (Herbst), and the confused flour beetle, *Tribolium confusum* Jacquelin du Val, in flour mills. Both *T. castaneum* and *T. confusum* are serious pests of flour mills worldwide. This thesis is a paper-style thesis with 6 chapters, Chapter 1 being

this introduction. Chapter 2 is a literature review of the biology and control of stored product insects, with special emphasis on *T. castaneum* and *T. confusum* in flour mills. Chapter 3 details research into the effects of disturbance, harbourage and food availability have on the fitness of *T. castaneum* and *T. confusum*. Chapter 4 discusses *T. castaneum* and *T. confusum* inside rollstands, i.e. machines used to grind flour in mills; the distribution of beetles inside rollstands is reported, as is the efficacy of pheromone trap sampling in determining which rollstands are most infested. Chapter 5 describes studies in trap efficacy between mill and laboratory strains of *T. castaneum* and *T. confusum*, and also details behavioural studies on the different strains and species. The final chapter, Chapter 6, is a general summary tying all the reported results together in the context of relevant literature.

Chapter 2: Literature Review

This literature review essay will discuss the biology and control of stored product insects, with special emphasis on *T. castaneum* and *T. confusum* in flour mills. An introduction to grain pests in Canada will be given, followed by a discussion of the biology of, and competition between, *T. confusum* and *T. castaneum*. Chemical and non-chemical methods of control will then be described, along with their advantages and disadvantages. The conclusion will give a rationale for the new research presented in this thesis.

The biology of stored product insects

There are six primary feeding strategies for insects found in stored products (Rees, 2004): commodity feeding, fungal feeding, predation, parasitism, scavenging and foraging. Commodity feeders are either primary pests, insects that can attack whole seeds, or secondary pests, insects that can only feed on damaged seeds. Fungal feeding is usually a supplemental type of feeding, providing insects with nutrients absent from the infested commodity. Some species however complete development on mould and cannot survive on grain alone. Predators and parasitoids found in stored products feed upon other insect pests and are therefore indicators of established pest infestations. Foragers include ants, cockroaches, and general predators like carabids (Rees, 2004).

Stored product insects generally have high rates of increase and can breed within a wide range of temperatures. The overall breeding range for these insects is 15-42°C with a typical optimal range between 25-33°C. Many species require little humidity to

reproduce, although grain moisture contents between 15-17% are typically necessary for these species to reproduce at optimal rates (Rees, 2004; Sinha and Watters, 1985).

Stored product insects in grain and grain processing facilities

There are many stored product insects associated with grain and grain processing facilities in Canada and the USA. To simplify the discussion, only the top fifteen species will be discussed here (Table 2.1). All of these insects are described as serious to severe pests and given a pest status ranking of at least 3 out of 4 by Rees (2004); according to Rees' scale, 1 is the least severe pest status and 4 is the most severe. These insects are all from the orders Lepidoptera and Coleoptera, are commodity feeders and are either primary pests, found in grain stores, or secondary pests, found in grain stores and/or grain processing facilities like flour mills (Rees, 2004). The fifteen insect pests of interest are grouped according to family in the following sections.

The lepidopteran pests lay their eggs within or near the commodity and the larvae feed upon it after emergence (Sinha and Watters, 1985). Lepidopteran adults are short-lived and do not feed (Rees, 2004). The coleopteran pests all lay their eggs within the commodity being attacked (grain/flour), and feed on the commodity both during larval stages and the long-lived adult stages (Rees, 2004).

Table 2.1. Major insect pests found in stored grain. Summarized from Rees (2004) and Sinha & Watters (1985).

Order	Family	Species	Common Name	Type of feeding
Coleoptera	Bostrichidae	*Rhyzopertha dominica* (F.)	Lesser grain borer	Primary
	Curculionidae	*Sitophilus granarius* (L.)	Granary weevil	Primary
		Sitophilus oryzae (L.)	Rice weevil	Primary
		Sitophilus zeamais Motschulsky	Maize weevil	Primary
	Laemophloeidae	*Cryptolestes ferrigineus* (Stephens)	Rusty grain beetle	Secondary
		Cryptolestes pusillus (Schönherr)	Flat grain beetle	Secondary
		Cryptolestes turcicus (Grouvelle)	Flourmill beetle	Secondary
	Silvanidae	*Oryzaephilus mercator* (Fauvel)	Merchant grain beetle	Secondary and mold feeder
		Oryzaephilus surinamensis (L.)	Saw-toothed grain beetle	Secondary and mold feeder
	Tenebrionidae	*Tribolium confusum* Jacquelin du Val	Confused flour beetle	Secondary
		Tribolium castaneum (Herbst)	Red flour beetle	Secondary
		Tribolium destructor Uyttenboogaart	False black flour beetle	Secondary
Lepidoptera	Gelechiidae	*Sitotroga cerealella* (Olivier)	Angoumois grain moth	Primary
	Pyralidae	*Ephestia kuehniella* (Hübner)	Mediterranean flour moth	Secondary and scavenger
		Plodia interpunctella (Hübner)	Indian meal moth	Secondary and scavenger

Grain storage pests

Lepidoptera: Gelechiidae

Sitotroga cerealella (Olivier), the Angoumois grain moth, is a primary pest of grain that can cause substantial direct crop loss (Hill, 1990; Rees, 2004) The maximum monthly rate of increase for this insect is 50 fold at its ideal conditions of 30°C, 70% humidity. Breeding can occur within the range of 16-35°C, humidity above 30%. Infestations produce excess heat and moisture, thereby supporting mold growth (Rees, 2004).

Coleoptera: Bostrichidae

Rhyzopertha dominica (F.), the lesser grain borer, is a primary pests of grain (Rees, 2004). Females of this species lay between 200 and 500 eggs (Hill, 1990). The maximum monthly rate of increase is 25 fold under ideal conditions (34 and 70% relative humidity). *Rhyzopertha dominica* can breed within 20-38°C, humidity above 30% (Rees, 2004).

Coleoptera: Curculionidae

Sitophilus granarius (L.), the granary weevil, *S. oryzae* (L.), the rice weevil, and *S. zeamais* Motschulsky, the maize weevil, are primary pests of grain. The maximum monthly rate of increase is 15 fold for *S. granarius* and 25 fold for *S. oryzae* and *S. zeamais* under ideal conditions (30°C, 70% humidity for all species). *Sitophilus granarius* cannot fly and is quite cold-hardy, capable of breeding in the range of 11-34°C

(humidity above 40%). For *S. oryzae* and *S. zeamais,* breeding can occur in the range of 15-34°C, humidity about 40% (Rees, 2004).

Grain storage and grain processing pests

Lepidoptera: Pyralidae

Ephestia kuehniella Zeller, the Mediterranean flour moth, and *Plodia interpunctella* (Hübner), the Indian meal moth, are secondary pests and scavengers, attacking grain and grain products (Rees, 2004). *Plodia interpunctella* is often associated with fungi in stored maize (Baker and Loschiavo, 1987). *Ephestia kuehniella* has a maximum monthly reproductive rate of 50 fold (at 25°C, 75% humidity) and *P. interpunctella* has a rate of 60 fold (at 30°C, 75% humidity). The breeding range for *E. kuehniella* is 12-30°C, humidity above 0%. *Plodia interpunctella* has a breeding range of 15-35°C, 25-90% humidity. *Ephestia kuehniella* and *P. interpunctella* can diapause in the larval stage (Rees, 2004).

Coleoptera: Laemophloeidae

Cryptolestes ferrigineus (Stephens), the rusty grain beetle, *C. pusillus* (Schönherr), the flat-grain beetle, and *C. turcicus* (Grouvelle), the flourmill beetle, are secondary pests in grain. Because of this, they usually occur in grain already attacked by *Sitophilus* spp. or *R. dominica* and can also be found in flour mills (Rees, 2004). *Cryptolestes ferrigineus* is capable of attacking grain that is even very slightly damaged (Hill, 1990). The maximum monthly rate of increase for these species is quite high, up to

60 fold at 35°C and 90% humidity. Breeding ranges are as follows: 20-42.5°C, 40-90% humidity for *C. ferrigineus*; 17.5-37.5°C, humidity above 50% for *C. pusillus*; 17.5-35°C, humidity about 50% for *C. turcicus* (Rees, 2004).

Coleoptera: Silvanidae

Oryzaephilus mercator (Fauvel), the merchant grain beetle, and *O. surinamensis* (L.), the saw-toothed grain beetle, are closely related species (Hill, 1990). They are secondary pests in grain and are therefore commonly found after infestations of *Sitophilus* spp. or *R. dominica*. They are also found in flour mills and feed on mold. The maximum monthly rate of increase is 20 fold for *O. mercator* and 50 fold for *O. surinamensis* (both at 30-32.5°C, 70% humidity). These two species have similar breeding ranges; 18-38°C, humidity about 10% for *O. mercator*; 20-38°C, humidity about 10% for *O. surinamensis* (Rees, 2004). *Oryzaephilus surinamensis* is fairly cold-tolerant, being capable of surviving below 0°C for many days (Hill, 1990).

Coleoptera: Tenebrionidae

Tribolium is the most encountered genus in stored products (Rees, 2004). Within this genus, *T. confusum*, *T. castaneum* and *Tribolium destructor* Uyttenboogaart are the most common, acting as secondary pests of grain. Of these, *T. castaneum* and *T. confusum* are the more frequently encountered. *Tribolium castaneum* and *T. destructor* are found in areas of grain storage and also flour mills while *T. confusum* is more restricted to flour mills (Rees, 2004). *Tribolium castaneum* and *T. confusum* are economically important pests in diverse climates while *T. destructor* is economically

important only in cooler areas, being unable to tolerate temperatures above 30°C for long periods (Rees, 2004).

Tribolium confusum and *T. castaneum* have among the highest maximum monthly rates of reproduction amongst stored product insects: 60 for *T. confusum* (32.5°C, 70% humidity) and 70 for *T. castaneum* (35-37.5°C, humidity above 70%). Breeding ranges for these two species are as follows: 22-40°C, humidity above 1% for *T. castaneum*; 19-37.5°C, humidity above 1% for *T. confusum*. *Tribolium destructor* has a lower rate of reproduction owing mainly to a longer developmental period (at least 44 days). *Tribolium destructor* also has a more restricted breeding range: temperature below 30°C, humidity above 10% (Rees, 2004).

Comparison between Tribolium confusum *and* Tribolium castaneum *biology*

Both *T. confusum* and *T. castaneum* are economically important pests worldwide (Rees, 2004). Their pest status along with their tolerance of inbreeding have made them popular test subjects in genetic studies, especially *T. castaneum* (Beeman and Brown, 1999; Lorenzen et al., 2005; Thomson and LaBonne, 1998; Richard et al., 2008). *Tribolium confusum* and *T. castaneum* probably originated from the regions surrounding India, Southwest Asia and the eastern Mediterranean (Good, 1936). These species has long been associated with stored products; *T. confusum* was found in a cereal jar in a pharoah's tomb dated 2500 BC (Sokoloff, 1974). The diets of both these species are varied, including a wide variety of foods of plant and animal origin (Good, 1936).

Both *T. confusum* and *T. castaneum* are similar in appearance. Indeed, *T. confusum* got its name because it had been confused with *T. castaneum* up until du Val

separated the two species (Good, 1936). Eye facets and antenna are important distinguishing characteristics. *Tribolium confusum* has only 1-2 eye facets laterally and has a 5-6 segmented club while *T. castaneum* has 3-4 facets and a three-segmented club (Bousquet, 1990). Also, *T. confusum* is usually slightly larger than *T. castaneum* (Sokoloff, 1974). Perhaps the largest difference is that *T. castaneum* flies when it is warm while *T. confusum* does not seem to fly at all (Rees, 2004).

Many *Tribolium* beetles, including *T. confusum* and *T. castaneum*, secrete defensive benzoquinones through abdominal and prothorax reservoirs. These quinones "condition" the flour in which these beetles live, discolouring it and making it smelly (Sokoloff, 1972). These quinones are a health concern since they are suspected carcinogens (Ladisch, 1953).

Tribolium confusum and *T. castaneum* males produce aggregation pheromones (Suzuki and Sugawara, 1979). Suzuki (1980) identified the aggregation pheromone for both *T. confusum* and *T. castaneum* as 4,8-dimethyldecanal. Suzuki and Mori (1983) report that 4R, 8R-(-)-4,8-dimethyldecanal is the only optical isomer of 4,8-dimethyldecanal that is as attractive to both *T. confusum* and *T. castaneum* as natural pheromone, and the isomer was attractive at doses as low as 1.0 ng. However, Suzuki et al., (1984) report that the natural pheromone of *T. castaneum* is probably a 4:1 mixture of both 4R, 8R-(-)- and 4R, 8S-(+)-4, 8-dimethyldecanal isomers, because this mixture was significantly attractive at doses as low as 0.1 ng. While Suzuki et al. (1984) suggest the aggregation pheromone of *T. confusum* also involves a mixture of the same two isomers of 4,8-dimethyldecanal, the ratio of the mixture may be different because *T. confusum* is less attracted to the 4R, 8S-(+) isomer than *T. castaneum*. *Tribolium castaneum* is

attracted to 100 ng doses of 4R, 8S-(+)-dimethyldecanal while 1000 ng is required for the same reaction from *T. confusum* (Suzuki and Mori, 1983; Suzuki et al., 1984).

Using scanning electron microscopy, Faustini et al. (1982) identified the femoral setiferous sex patch present on *T. castaneum* males as the site of aggregation pheromone production, with females and males responding to this pheromone within a day after eclosion. Behavioural responses to the pheromone include extension of the prothoracic leg, protraction of the antenna and zig-zag movement towards the source of the pheromone (Faustini et al., 1982). Age does not greatly affect pheromone production; Hussain et al. (1994a) report no differences in production levels during first month and Faustini et al. (1982) show that the pheromone produced by 150-day-old males is still attractive to females. Food does affect production however since pheromone production drops 90% when beetles are starved (Hussain et al., 1994a).

Larvae of *T. castaneum* are attracted to synthetic pheromone (4,8-dimethyldecanal, racemic mixture) and repelled by synthetic 2-methyl-1,4-benzoquinone, with early instars reacting more strongly than late instars in both cases (Mondal and Port, 1984a; Mondal and Port, 1984b). When *T. castaneum* larvae are exposed to benzoquinones and aggregation pheromone, the sex ratio is altered from the usual 1:1 (Mondal, 1987). Larval exposure to 2-methyl-1,4-beonzoquinone results in more males than females after eclosion, but the sex ratio becomes biased towards female in the presence of 4,8-dimethyldecanal. Both pheromone and quinone together results in no alteration of the sex ratio. Mondal (1987) suggests that the sex ratio may change because of differential mortality or because these compounds affect the sex determination mechanisms in *T. castaneum* in an unknown manner.

Tribolium castaneum generally develops faster than *T. confusum* due to shorter durations in the egg and pupal stages (Park, 1948). The time to develop from egg to adult changes depending on temperature, humidity and genetic strain (Sokoloff, 1974). The egg and pupae stage are the shortest stages with the larval stage being the most variable. The number of larval instars varies from 5-9 in *T. castaneum* and 5-11 in *T. confusum*, depending on nutritional status and water content of food (Sokoloff, 1974).

Ziegler (1976) reports that emigration patterns of *T. castaneum*, along with a review on the literature of this beetle, show *T. castaneum* to be a primary colonizer that widely disperses, depletes resources then moves to new areas. *Tribolium castaneum* adults that disperse the most develop the fastest and lay the most eggs, especially in the presence of high population densities (Cox and Collins, 2002). In contrast, T. *confusum* is more of a secondary colonizer, preferring previously exploited territories (Ziegler, 1976). These species differences can be observed in terms of attraction to conditioned medium. *Tribolium castaneum* prefers fresh flour and is repelled by conditioned flour (Sokoloff, 1974; Ghent, 1963). In contrast, *T. confusum* shows a preference for conditioned flour (Ghent, 1963), dispersing less from the surface of flour as it became more conditioned (Ogden, 1970) and selecting conditioned flour over fresh (Naylor, 1959; Ghent, 1963).

Tribolium castaneum is generally more sensitive to environmental conditions than *T. confusum* (Park, 1955). For example, *T. castaneum* displays greater increases and decreases in population size than *T. confusum* when humidity fluctuates, always having the greatest population sizes under increased humidity (Park, 1954). Oviposition rates in both *T. confusum* and *T. castaneum* are influenced by many environmental factors including temperature, relative humidity, availability of food and population densities

(Sokoloff, 1974). When presented with patches of flour that differ in size, *T. castaneum* can adjust their oviposition rates in each patch to maximize net fitness (Campbell and Runnion, 2003).

Competition studies

Park (1948) reviews the reasons for competition between *T. confusum* and *T. castaneum*: reproductive patterns are quite similar, they are almost the same size, they have similar taxonomy and they exploit the same source. Park authored three influential studies between the years 1948 and 1957, exploring the effects of competition between *T. confusum* and *T. castaneum*. In all these studies (Park, 1948; 1954; 1957) the two species never coexist indefinitely. While often mixed-species cultures exist for many years, eventually either *T. confusum* or *T. castaneum* eliminates its competitor and the culture becomes a single-species culture. As well, the victor in competition depends on many factors and cannot always be predicted beforehand.

When *Adelina tribolii* Bhatia, a coccidian parasite of *Tribolium* spp., is added to cultures containing equal beginning densities of both *T. confusum* and *T. castaneum*, *T. confusum* usually eliminates its competitor (11 out of 15 repetitions). When these cultures contain *T. confusum*:*T. castaneum* ratios of 1:3 and 3:1 respectively, *T. confusum* continues to be the usual victor (11 out of 15 times with more initial *T. confusum*; 15 out of 15 times with more initial *T. castaneum*). However, *T. castaneum* wins more often than *T. confusum* under all conditions when *A. tribolii* is removed (Park, 1948).

In Park (1954), mixed-species cultures of *T. confusum* and *T. castaneum* are placed at equal densities, in three different temperatures (24°C, 29°C and 34°C) and two

different humidities (30% and 70%; Table 2.2). Only two conditions result in consistent victories for a particular species; *T. confusum* always wins at 24°C, 30% humidity and *T. castaneum* always wins at 34°C, 70% humidity. *Tribolium confusum* always wins at 24°C, 30% humidity because *T. castaneum* cannot survive in those conditions over long periods of time (Park, 1954). *Tribolium castaneum* is more sensitive to low humidities than *T. confusum*, winning less competitions at 30% than 70%. *Tribolium castaneum* is also more tolerant of high temperatures than *T. confusum,* generally winning more competitions as temperature increases (Park, 1954.)

Tribolium confusum wins 14% of the time under 24°C and 70% humidity (Park, 1954; Table 2.2). To see the effects of natural selection on competition between these two species, Park and Lloyd (1955) placed the progeny of these winning *T. confusum* in competition against *T. castaneum* taken from a stock culture. If natural selection plays a role in competitive outcome, there should be significantly more *T. confusum* wins in this study than there were in Park (1954). This was not the case; the percentage of *T. confusum* and *T. castaneum* victories are the same as before. Park concludes that natural selection resulting from previous competitions cannot be used to judge the outcome of subsequent interspecies competitions.

Table 2.2. Results of interspecies competition between *Tribolium confusum* and *Tribolium castaneum* under varying conditions of temperature and humidity. Summarized from Park (1954).

Humidity (% RH)	Temperature (°C)	% of *Tribolium confusum* wins	% of *Tribolium castaneum* wins
30	24	100	0
	29	87	13
	34	90	10
70	24	71	29
	29	14	86
	34	0	100

In Park (1957), initial densities of *T. confusum* and *T. castaneum* were varied by adding different numbers of *T. confusum* and *T. castaneum* eggs to vials of flour. Equal numbers of *T. confusum* and *T. castaneum* eggs were added together in two additional treatments, but one species' eggs were added six days before the others. Temperature and humidity were held constant at 34°C, 70% humidity because this condition results in the consistent victory of only one species, *T. castaneum* (Park, 1954). Since one species is completely favoured over the other, the effect of initial densities of both species on competitive outcome is easier to discuss.

Tribolium castaneum is always victorious under all but one condition (90 *T. confusum* eggs and 10 *T. castaneum* eggs added together; Table 2.3). The reason for this seems to be female mortality and fecundity; female *T. confusum* have diminished capacities for reproduction in the face of *T. castaneum* competition and female *T. confusum* die before the males in this study. Once again, one species always eliminates the other.

Table 2.3. Results of interspecies competition between *Tribolium confusum* and *Tribolium castaneum* under varying intial egg densities. Summarized from Park (1957).

Number of initial *T. confusum* eggs	Number of initial *T. castaneum* eggs	% of *T. castaneum* wins	% of *T. confusum* wins
90	10	64	36
70	30	100	0
50	50	100	0
30	70	100	0
10	90	100	0
50	50[1]	100	0
50[2]	50	100	0

1. *T. castaneum* eggs added 6 days after *T. confusum* eggs
2. *T. confusum* eggs added 6 days after *T. castaneum* eggs

Leslie et al. (1968) varied ratios of *T. confusum* and *T. castaneum* adults introduced into mixed-species cultures. In contrast to the other competition studies already discussed, one of the cultures in Leslie et al. (1968) remained a mixed-species culture over the course of the 960-day study. Edmunds et al. (2003) analysed the population dynamics of this culture and developed a competition larvae, pupae and adult population model which permits these two species to live together indefinitely, remaining in strong competition with each other and experiencing periodically oscillating populations. While Park's studies strongly indicate that *T. confusum* and *T. castaneum* cannot exist together indefinitely when competing for a limited resource, and the mixed-species culture mentioned was not examined after the study was over, Park does allow for the possibility of coexistence (Park, 1957). Whether these two species can coexist under any conditions remains to be seen.

Tribolium species are cannibalistic, with genetic differences in cannibalism rates occurring both between and within different strains (Stevens, 1989; Park et al., 1968).

There are six different cannibalistic pathways in *Tribolium*: adults eating eggs, adults eating pupae, larvae eating eggs, larvae eating pupae, larva eating larva and adults eating larva. The two pathways involving cannibalism of larvae are the least significant (Park et al., 1974). Cannibalism, in particular larval-egg predation, helps regulate single-species populations (Hastings and Costantino, 1987). Cannibalism also plays an important role in interspecies competition, with pupal predation of particular interest. Both adult *T. confusum* and *T. castaneum* prefer to prey on the competing specie's pupae, with *T. castaneum* showing the highest interspecific preference and the highest rate of interspecific pupal predation (Park et al., 1968). *Tribolium castaneum* shows an increased preference for *T. confusum* pupae as the number of pupae from both species increases, while *T. confusum* displays less of a preference as the number of overall pupae increases (Park et al., 1968).

The damage associated with stored product insects

Insects can damage foodstuffs such as grain both directly (feeding) and indirectly (contamination). In terms of direct insect grain feeding, Gecan et al. (1980) report that insect-damaged kernels were present in 35% of the 1200 American wheat samples they analysed, and overall the mean weight of such damaged kernels was 70 mg per 100 g. Insect feeding not only causes mechanical damage to kernels but also can result in damage to kernels due to starch breakdowns and heat production (Snelson, 1987). Reduced seed germination, protein content and amino acid levels are all results of insect infestations (Baker and Loschiavo, 1987).

Tribolium castaneum larvae are estimated to attack 4 wheat grains each, consuming almost all the germ of attacked grains (White and Lambkin, 1988). *Tribolium castaneum* is a secondary pest of grain however, eating only grain that has already been injured (Rees, 2004). Because it cannot eat whole (i.e. intact and uninjured) grain, Subramanyam and Hagstrum (2000) suggest that *T. castaneum* does not need to be included in multi-species models that calculate weight loss of stored grain due to insect feeding.

In terms of indirect damage through contamination, many stored product Lepidoptera present in grain (i.e. *E. kuehniella*) contaminate grain with silk webs (Rees, 2004). As well, there are legal limits on the number of insects that can be found in grain, and exceeding these limits include rejection of the commodity and additional fines/other financial penalties (Snelson, 1987). Eye irritation and allergic reactions can result from high levels of insect fragments (Snelson, 1987) and such allergens are often not completely destroyed through heat exposure during processing (Phillips and Burkholder, 1984). Legal action and consumer complaints have arisen from health complaints over such insect/mite contamination (Phillips and Burkholder, 1984).

Damage in flour mills due to T. castaneum *and* T. confusum

The amount of direct flour loss that can be attributed to *T. castaneum* and *T. confusum* in flour mills is unclear. Both *T. confusum* and *T. castaneum* are continuous feeders (Sokoloff, 1974) and it is estimated that *T. castaneum* eat approximately 13 mg of flour in the larvae stage and 315 mg in the adult stage (Subramanyam and Hagstrum, 2000). There are no similar studies on *T. confusum*, but values should be similar given the

insects have similar longevity and size. It is doubtful that this consumption rate affects the overall amounts of flour produced by flour mills, since the amounts eaten are small.

There are legal limits on insect fragments present in flour and these limits are more stringent then those imposed on grain. There is zero tolerance for live/dead insects in flour and only 10 insect parts per 50 g flour are permitted (Government of Canada: Health Protection Branch, 1999). As with grain, the costs associated with exceeding limits are quite high, involving possible fines and penalties. There is also the threat of legal action being taken against the company, or lost business.

Consumers tend to be even less tolerant of insect contamination than government and the business sector; to quote Snelson (1987, p.4): "If there were to be a statement on a package of flour to the affect that 'this flour contains fewer than 10 insect fragments per 100g', it would have an adverse effect on the consumer". Any evidence of insect contamination in food may lead to negative publicity for the company producing the product and resulting economic losses. High consumer standards, in addition to commodity damage, loss of quality and health concerns, make it necessary to control insect populations in food processing facilities as tightly as possible.

Insecticides to control stored product insects

Fumigants and contact insecticides are the main methods used to control stored product insects. Fumigants are gases that diffuse throughout large areas, providing overall control of pest populations (Bond, 1984). Contact insecticides are applied to surfaces and can be used for control over small or large areas (Snelson, 1987).

Fumigants

Fumigants usually exert their toxic effects through the respiratory system of insects (Bond, 1984). Methyl bromide is an especially important and popular fumigant, used widely in flour mills, feed mills, ship holds and for quarantine treatments (Fields and White, 2002). Because it is an ozone depleter (Ozone Secretariat, 1992), its use is currently being phased out and investigation into alternative fumigants is ongoing. In this paper, methyl bromide will be discussed first, followed by possible alternative fumigants.

Methyl bromide

Methyl bromide (CH_3Br) is a naturally occurring compound produced by maine plankton and found in the world's oceans (Mano and Andreae, 1994). Methyl bromide is a neurotoxin which exerts its effects through reaction with protein sulfhydryl groups (World Health Organization and United Nations Food and Agriculture Organization, 1978). It is commonly used in the fumigation of grain and flour mills, along with soil and quarantine fumigations (Bond, 1984; Fields and White, 2002). Since methyl bromide is odourless, chloropicrin can be added as a warning agent (Bond, 1984).

Methyl bromide works quickly (with insects usually dying within 24 hours of exposure) and it is effective against a wide range of pests including insects, nematodes, mites and microflora (Fields and White, 2002). This fumigant also has the following advantages: it penetrates deeply into commodities; it works quickly; it is noncorrosive, non-flammable and non-explosive under normal conditions; its vapours dissipate quickly so that bulk commodities can be handled safely and shortly after fumigation; plants can often tolerate its use and it has a low boiling point (3.6°C) and therefore can be used at

low temperatures (Bond, 1984; Fields and White, 2002). It also does not tend to taint the commodity being treated (Fields and White, 2002). El-Lakwah et al. (1990) report that 0% of wheat and flour samples they examined in Egypt had above-acceptable residual levels. Methyl bromide is also relatively cheap to use, costing approximately $7.40 US per 1000 cubic feet of treated area as of 1997 (United States Environmental Protection Agency, 1997).

While methyl bromide is widely used, alternatives are being sought because this fumigant is an ozone-depleter. Most ozone (about 90%) is located in the stratosphere, approximately 10-50 km above the surface of the earth; this area of ozone concentration is commonly referred to as the "ozone layer". The ozone layer is necessary to filter out harmful UV-B light that is harmful to all living cells (Ozone Secretariat, 2000). Evidence that the ozone layer was being destroyed came in 1985, with the report of a "hole" in the ozone layer over Antartica (Farman et al., 1985). To avoid further destruction of the ozone layer, the Montreal Protocol on Substances that Deplete the Ozone Layer was formed in 1987. As of 8 November 2006, only five states worldwide had not yet ratified these ozone treaties; these states are Iraq, Timor Leste, Andorra, Holy See and San Marino (Ozone Secretariat, 2006).

Chlorofluorcarbons (CFCs) were shown to destroy ozone (O_3) by Molina and Rowland (1974). CFCs are highly stable and can travel into the ozone layer. The reactions between chlorine from CFCs and ozone are described below; bromine from methyl bromide reacts with ozone in a similar manner (Ozone Secretariat, 2000).

$Cl + O_3 \rightarrow ClO$ (unstable) $+ O_2$

ClO (unstable) $+ O \rightarrow Cl$

Repeat: $Cl + O_3 \rightarrow ClO$ (unstable) $+ O_2$ etc.

Because chlorine acts as a catalyst, one chlorine atom can destroy up to 100,000 ozone molecules. Bromine atoms reacts with ozone even more violently, destroying up to 40 times as many ozone molecules as chlorine (Mano and Andreae, 1994). It is believed that 5-10% of stratospheric ozone loss seen in 1994 was due to methyl bromide alone (Mano and Andreae, 1994). Both bromine and chlorine also react together in a synergistic manner to deplete ozone; the rate-limiting step in this reaction is $ClO + BrO \rightarrow Cl + Br + O_2$ (McElroy et al., 1986).

In the Copenhagen amendment to the Montreal Protocol, methyl bromide fumigation was scheduled to be phased-out (Ozone Secretariat, 1992). Developed countries were to phase-out non-critical use of this fumigant by 1 January 2005, with developing countries given a deadline of 2015 (Technology and Economic Assessment Panel, 2004). Flour mills are still using this fumigant today under the critical use exemptions from the Montreal Protocol (Technology and Economic Assessment Panel and Methyl Bromide Technical Options Committee, 2006).

Finding new alternative fumigants for methyl bromide is difficult because of the economics involved; registration of a new fumigant is costly, the fumigant market is a small one and some older fumigants need to be reregistered for use (Bell et al., 1996). Still, there are two fumigants, phosphine and sulfuryl fluoride, that are currently considered viable methyl bromide alternatives for flour mills.

Phosphine

Phosphine gas, with the chemical name of hydrogen phosphate (PH_3), has been used to control stored product pests since the 1930s. It is believed to work on the biological redox system, especially interfering with the mitochondrial electron transport chain (Chaudhry, 1997). It has a low boiling point of -87.4°C, making it useful for fumigation when temperatures are low (Bond, 1984). Phosphine leaves no toxic residues behind and it does not adversely affect viability of seeds (Chaudhry, 1997). It is also relatively cost effective at approximately $8.25 US per 1000 cubic feet for aluminum phosphide tablets and $11.10 US per 1000 cubic feet for magnesium phosphide plates as of 1997 (United States Environmental Protection Agency, 1997).

Phosphine became widely used in grain with the development of metal phosphide formulations (i.e. aluminum phosphide, AlP, or magnesium phosphide, Mg_3P_2); such formulations react with moist air to release gas. Phosphine gas diffuses rapidly in air because of its similar density to air, usually distributing itself naturally throughout the area undergoing fumigation. Aluminum phosphide is used in stored grain while magnesium phosphide can be used for space fumigations, i.e. warehouses (Fields, personal correspondence). Cytec Industries Inc. has patented a mixture of 2% phosphine (by weight) in carbon dioxide under the trademark of ECO_2FUME, and has registered it for use in Canada, USA and Europe (Cytec Canada Inc., 2006). Using carbon dioxide instead of air to diffuse phosphine gas makes it possible to increase the amount of phosphine used (Fields, personal correspondence).

Longer fumigations with low concentrations of phosphine are more effective at controlling insects than shorter fumigations with higher concentrations (Hole et al.,

1976). Some insects protect themselves against high concentrations of phosphine by entering into a narcosis; this may protect insects by decreasing respiration and metabolic rates (Chaudhry, 1997). Eggs and pupae are usually more difficult to kill with phosphine than larvae and adult stages (Chaudhry, 1997), but increased exposure times result in insects reaching a developmentally-susceptible stage during fumigation (Hole et al., 1976). Adding heat to a phosphine fumigation helps decrease the fumigation time necessary because it decreases developmental time (Hole et al., 1976).

Phosphine gas has its disadvantages. It does not work against some destructive stored product mites, including *Cheyletus eruditus* (Schr). It is slow-acting; exposure times of three or more days may be required, depending on the temperature. It is also flammable above 1.8% concentration by volume (in air) and corrosive to metals, with higher humidities and temperatures leading to higher corrosive rates (Bond, 1984; Fields and White, 2002).

There are also concerns over health effects related to long-term exposure to phosphine. In a correlation study, Garry et al. (1989) report that chromosomal rearrangements were present in fumigant and chromatid deletions and gaps were observable shortly after exposure. In response to these types of reports, the USA, Australia, UK and some other European countries increased restrictions on the use of phosphine as a fumigant (Bell, 2000).

Significant insect resistance to phosphine has developed worldwide, to the point where it is an economic concern (Bell, 2000). Within populations of any given insect, there are natural genetic variations in susceptibility to an insecticide such as a fumigant. Because of this, some insects may survive fumigation. Surviving insects produce progeny

that inherit their parents' high tolerance of the fumigant. If the same fumigant is used repeatedly, the frequency of resistance in the population increases and renders the fumigant useless (Bond, 1984). The exact mechanism of phosphine resistance in insects is not known, but there may be a system in the tracheal membranes that excludes phosphine before it reaches the insect's cellular target sites (Chaudhry, 1997).

Rajendran (1994) proposes that increasing overall concentrations of phosphine during a fumigation may prevent the development of resistance, since fumigant concentrations naturally decrease over the course of a fumigation and lowered concentrations may lead to higher survival rates and increased resistance. Chaudhry (1997) reports that methylphosphine (CH_3PH_2), used alone or in combination with phosphine, may be effective against phosphine-resistant strains. Adding carbon dioxide may help by reducing the time needed for fumigation; El-Lakwah et al. (1989) report that while only 50% of *T. castaneum* adults die within 72 hours of exposure to phosphine gas (at 28°C), adding 20% CO_2 leads to 100% mortality within the same time frame.

Combination treatments involving phosphine, supplemented CO_2 and heat have proven successful, with a reported 40 successful fumigations between 1991 and 2002 (Fields and White, 2002). Mueller (1994) reports 100% insect mortality in three separate mills with 24 hours of exposure to 65-100 ppm phosphine, 4-6% CO2 and 32-37°C. Mueller was unable to give exact figures on fumigation costs, but Fields and White (2002) state that this type of combination treatment costs 20-50% more than a methyl bromide fumigation. This combination treatment has been used for several years in US flour mills (Mueller, 1993) and has recently been approved for use in Canadian flour mills (Fields, personal communication).

Sulfuryl fluoride

Sulfuryl fluoride (SO_2F_2, boiling point -55.2°C) is a structural fumigant that has been used successfully to control termites (Bond, 1984). Dow AgroSciences has trademarked a sulfuryl fluoride fumigant under the name of ProFume and registered it for use in Canada, USA and Europe (Dow Agrosciences Canada Inc., 2006). ProFume has recently been approved for use in empty cereal grain mills, empty storage facilities and empty food processing plants (Pest Management Regulatory Agency, 2007), with approval for use on food pending (Fields, personal communication). It is an odourless gas that disperses rapidly, penetrates well and is non-flammable (Bond, 1984).

Small (2007) compared methyl bromide and sulfuryl fluoride fumigations done in different flour mills, by monitoring populations of *Tribolium* spp. and *E. kuehniella* with pheromone traps before and after fumigations. Small (2007) reports both methyl bromide and sulfuryl fluoride were equally effective in controlling insect populations. Tsai et al. (2006) reports similar results. Tsai et al (2006) used bioassays during fumigations and report sulfuryl fluoride fumigation causes 100% mortality for larval, pupal and adult stages of *T. castaneum* and 99.3% survival of *T. castaneum* eggs. Methyl bromide fumigation kills 100% of egg, larval and adult stages, with over 99.6% of pupae dying as well.

The egg mortality rate for sulfuryl fluoride may be cause for concern. In general, this fumigant is known for being very toxic to all stages of insects except eggs (Bond, 1984). In a possible solution to this problem, Solvay Fluor und Derivate GmbH has patented the idea of combining sulfuryl fluoride with an ovicidal gas such as hydrocyanic acid (Baeumert and Belt, 2005). In addition to increasing egg mortality rates during

fumigation, the concentrations of each of these fumigants can be decreased when they are used together due to their synergistic interaction (Baeumert and Belt, 2005).

Contact insecticides

There are several types of contact insecticides, including organophosphates, carbamates, pyrethroid pesticides and insect growth regulators. The organophosphates act on the nervous system, disrupting acetycholinesterase. Pyrethroid pesticides are insecticides that have similar chemical structures to pyrethrin, a natural compound found in chrysanthemums; they act on the nervous system as well. Insect growth regulators mimic natural hormones (juvenile hormone, ecdysone) to disrupt molting, or interfere with chitin synthesase to disrupt chitin synthesis (Ware, 1999).

Organophosphates

Some of the world's most widely-used insecticides are organophosphates. However, these chemicals can have severe effects on humans. Paralysis and death can result from exposure to high doses, and headaches and nausea have been reported at lower doses. Citing these reports, the United States Environmental Protection Agency is currently reviewing all organophosphates, including the ones discussed here (United States Environmental Protection Agency, 2006).

Malathion is one of the world's most widely-used insecticides and has been for decades (Sinha and Watters, 1985; Snelson, 1987). It is also less potent than the other organophosphates; high doses of it are required to control *R. dominica* and it does not work effectively against many moths (Snelson, 1987). Malathion resistance has become a

large problem, diminishing the effectiveness of this insecticide in many regions of the world. As an example of the extent of this problem, 87% of *T. castaneum* strains collected from 78 countries showed malathion resistance as of 1972 (Sinha and Watters, 1985). Many malathion-resistant insects showed cross-resistance to other organophosphates as well by 1980 (Sinha and Watters, 1985).

Pirimiphos-methyl was introduced to the market in the 1960s (Snelson, 1987). It acts quickly on a variety of pests, especially mites (Wilkin and Hope, 1973), and is effective against many strains of *T. castaneum* that are resistant to both lindane and malathion (Pieterse et al., 1972). It is also more effective at controlling *T. confusum* than most other organophosphates, requiring lower doses than the others at both low and high temperatures (O'Donnell, 1980). Some strains of *R. dominica* are resistant to it however (Snelson, 1987).

Three other organophosphates are dichlorvos, chlorpyritos-methyl and fenetrothion. Dichlorvos (also referred to as DDVP) was introduced as an insecticide in 1955. It is effective against most stored product insects, including moths and various species of larval within grain. Chlorpyrifos-methyl is effective on a variety of pests, has moderate lasting power and is less toxic than many organophosphates. There are resistant strains of *R. dominica* however. Fenetrothion is also relatively non-toxic and also has the same resistance problems with *R. dominica* as the others. It can be combined with pyrethroids to increase efficacy (Snelson, 1987).

Pyrethroids

Pyrethrum is a natural product that has insecticidal activity. Pyrethroids, i.e. synthetic pyrethrin-like compounds, have a low acute oral toxicity, rapid onset of action and are effective against a wide variety of insects. Recently developed pyrethroids such as deltamethrin also work at very low doses, do not break down when exposed to sun (a problem with natural pyrethrum) and have residual action for up to 10 days (Ware, 1999). When used in a spray form, pyrethroids encourage the rapid dispersal of insects, thereby increasing the insects' contact with treated surfaces (Snelson, 1987). It is difficult to encourage commercial involvement with pyrethroids however because botanical compounds cannot be patented and therefore cannot make much money (Bell et al., 1996). They are also expensive (Bell et al., 1996) and they do not have much residual action, making frequent reapplication necessary (Snelson, 1987).

Insect growth regulators

Insect growth regulators (IGRs) are not in common use yet and many products are still in the developmental stages. IGRs have the advantage of acting selectively on insects by mimicking hormones not found in humans. This makes them safer than other insecticides. There are three main classes of IGRs: chitin synthesis inhibitors, juvenile hormone analogues and edcysteroid agonists. Chitin synthesis inhibitors work by interfering with the creation of chitin, a crucial part of an insect's exoskeleton. Juvenile hormone analogues disrupt molting in insects through mimicking juvenile hormone. Edcysteroid agents also interfere with molting, but by mimicking ecdysone, i.e. molting hormone (Oberlander and Silhacek, 2000).

The mechanisms of action for juvenile hormone and edcysteroid agents merit further elaboration here. Both ecdysone and juvenile hormone are critical in the regulation of insect development. Ecdysone initiates molting and metamorphosis. Juvenile hormone mediates the insect's response to ecdysone, usually suppressing molting behaviour. There are several forms of juvenile hormone but juvenile hormone III is the most commonly-occuring form in insect species (Chapman, 1998).

If juvenile hormone is applied to hemimetabolous insects during critical larval stages, the insect does not moult into an adult. For holometabolous insects such as *Tribolium* spp., transitions from larvae-pupae and pupae-adult can only occur in the absence of juvenile hormone (Chapman, 1998). Applying juvenile hormone to immature insects therefore prevents them from becoming viable adults, and this interference with the regular growth pattern can cause death. Also, the application of juvenile hormone to females can result in unviable eggs (Snelson, 1987). Edcysteroid agents work in the opposite fashion, causing the insect to moult early and ultimately die (Collins, 2006). Juvenile hormone analogues have been used commercially (Technology and Economic Assessment Panel and Methyl Bromide Technical Options Committee, 2006) while commercially viable edcysteroid agents are still being developed.

Other contact insecticides

Inert dusts

Inert dusts can be defined as "all dry powders of different origins that are chemically unreactive in nature" (Subramanyam et al., 2000, p. 321). While there are

many different inert dusts that have insecticidal properties (Subramanyam et al., 2000), diatomaceous earth is the most commercial viable inert dust. Diatomaceous earth is a natural product consisting of fossilized diatoms. These diatom deposits, dating back to the Cenozoic era, primarily contain silicon dioxide secreted by the diatoms during their lifetime (Subramanyam et al., 2000). Diatomaceous earth causes insect dessication by absorbing wax from the cuticle. It has a low mammalian toxicity and, like all inert dusts, it is quite stable (Fields, 1999). As of 2000, there were over 40 different diatomaceous earth dusts registered in the United States, many of which are registered for use on grain (Subramanyam et al., 2000).

Insect resistance has been reported with diatomaceous earth, despite its natural status. Rigaux et al. (2001) studied fourteen different strains of resistant *T. castaneum* to see the mechanisms underlying this resistance. They report that tolerant strains are less susceptible to dessication because they loose water at a lower rate (both with and without exposure to diatomaceous earth). Tolerant *T. castaneum* strains also move at a slower rate and avoid wheat treated with diatomaceous earth, resulting in less exposure to this contact insecticide.

Pea protein

Peas are insecticidal to several stored product insects. Fields et al. (2001) report that pea protein is repellent to five different grain insects, including *T. confusum* and *T. castaneum*, and Hou et al. (2004) found protein-rich pea flour both toxic and repellent to *S. oryzae*, *C. ferrugineus* and *T. castaneum*. Protein-rich pea flour does not have adverse

effects on two *S. oryzae* and *C. ferrugineus* parasitoids, so both parasitoids and pea flour can be used together to reduce pest populations (Hou et al., 2004).

Advantages and disadvantages of insecticide use

There are several advantages associated with using insecticides, especially fumigants. As discussed, fumigants control a wide range of pests (insects, mites etc.), making them useful in a variety of industries no matter what pests may be present. Fumigations usually occur only once or twice a year (Fields and White, 2002) and fumigations can be booked in advance so that they occur during long-weekends and do not interfere with the regular production schedule (Fields, personal correspondence). The infrequency of fumigations, the broad spectrum of their activity and the ability to schedule fumigations around production schedules makes fumigation a cost-effective and time-saving means of pest control. Contact insecticides have many of the same advantages.

Insecticide use has serious repercussions however. Insect resistance to many insecticides, even natural substances like diatomaceous earth, has been reported. Insects are also capable of behavioural avoidance of pesticides; *T. castaneum* avoids diatomaceous earth (see above) and pyrethroids, among others, and may also secrete quinones to reinforce avoidance behaviour (Prickett and Ratcliffe, 1997). Cross-resistance to other insecticides often makes it difficult to find an alternative once resistance has made an insecticide useless, and alternative insecticides are often more expensive (Sinha and Watters, 1985). Environmental effects are also of concern. Using

fumigants in urban-situated processing facilities is becoming increasingly difficult due to municipal ordinances (Dowdy and Fields, 2002).

Because of health and environmental concerns, insecticide use is becoming increasingly regulated. The improper use of an insecticide can end up being quite costly. A famous example of this occurred in the 1990s, when Fumicon Inc., under a contract from General Mills, sprayed imported oats with the insecticide chlorpyrifos (trademark Dursban). Chlorpyrifos was not approved for food use, but it is chemically similar to an insecticide that was approved for such use (chlorpyrifos-methyl, trademark Reldan 4E). (Chlorpyrifos-methyl is no longer approved for food use either, having recently undergone a product cancellation; United States Environmental Protection Agency, 2007). When the Food and Drug Agency found trace amounts of chlorpyrifos in the breakfast cereal Cheerios, all product was recalled across the United States. In addition, General Mills shut down production to thoroughly clean their production factories and almost 500,000 tonnes of oats were quarantined. This case cost General Mills between 63 and 88 million dollarsUS (Feder, 1994). The man responsible for illegally spraying Dursban was sentenced to five years in prison, three years of supervised release and 200 hours of community service (Anonymous, 1995).

Consumer demands may be the most compelling reason why it is necessary to start employing non-chemical control alternatives on a larger scale. In 1991, a pesticide review panel asked Ottawa to enact plans to reduce overall pesticide use and put more money into researching alternative control methods; the panel cited increasing public concern over health and environmental effects (Mittelstaedt, 1991). Papers such as Fields et al. (2001) have also cited public demands as a primary reason for non-chemical control

methods. The next section of this paper will discuss physical control methods currently available to control insects.

Non-chemical methods of control

Physical control methods

Physical control methods rely on the manipulation of the stored product environment to decrease or eliminate pests (Fields and White, 2002). Extreme temperatures, i.e. heat or cold treatments, subject insects to conditions in which they cannot survive. Impact devices cause direct injury to insects. Sanitation directly removes and excludes insects. Heat and cold treatments are discussed here along with impact devices. Sanitation is discussed in a future section related to integrated pest management.

Heat

Controlling insects through exposure to high temperatures is not a new idea. This technique was first used in 1762 and was a common method of insect control in parts of the United States in the first part of the 20th century. Like other physical control methods, no residues are left in treated commodities and the human health risks are lower than those associated with insecticide use. It is also relatively cost-effective; on a yearly basis it costs about the same as fumigation with methyl bromide, excluding the cost of the initial modifications needed and the purchase of heaters. No insect resistance has been reported to heat treatments (Fields and White, 2002).

Temperatures above 35°C are usually lethal for stored product insects and the higher the temperature, the faster death occurs. It is unclear how insects die at high

temperatures; denaturation of proteins, pH changes and water stress probably all play a role (Fields, 1992). For effective control of insects, 50-57°C for 24-36 hours is usually recommended. Performing such a heat treatment for 24-36 hours is necessary to ensure all parts of the treated building reach 50°C (Fields and White, 2002). Upon exposure to 50°C, insect death occurs in minutes (Fields, 1992).

Building modifications are necessary if heat treatments are to take place. Severe heat can damage equipment and warp plastic, so anything that is heat-sensitive needs to either be removed or protected through enclosed application of cool air. Even with these modifications, damage to equipment and the structure of the building may occur if heat is not properly circulated (Imholte and Imholte-Tauscher, 1999). Non-uniform heating can also result in a greatly reduced insect mortality rate (Mahroof et al., 2003).

As mentioned previously, heat increases the efficacy of phosphine/CO_2 fumigations. Using diatomaceous earth during a heat treatment also increases insect control; insects increase their rate of movement in response to heat, resulting in increased contact to the diatomaceous earth. Diatomaceous earth can also control any insects that remain after heat treatment, since it has residual activity (Dowdy and Fields, 2002).

Young *T. castaneum* larvae and pupae are more tolerant of heat than adults, late-instars and eggs (Mahroof et al., 2003; Mahroof et al., 2005). Heat shock proteins, proteins which help protect an insect's biological system against negative effects of excess heat (Chapman, 1998), may play a role. HSP70 (heat shock protein 70) levels are increased in young *T. castaneum* larvae upon exposure to 40°C while eggs have reduced levels and other stages show no differences (Mahroof et al., 2005).

Cold

Stored product insects cannot reproduce below 15°C and they die between -10°C and -18°C (Fields, 1992). An exception to this is *E. kuehniella* which can reproduce at 12°C, though survival rates are low and development very long (Hill, 1990). As with high temperatures, the exact cause of death at temperatures well below 0°C is not known. Membrane fluidity, enzyme inactivation and ice formation are some of the possible mechanisms (Fields, 1992). Low relative humidities can decrease the necessary exposure time significantly (Fields, 1992).

The amount of insect control obtained from cold treatments depends on a number of factors. Previous exposure to lowered temperatures cause an insect to become "cold acclimatized" and more able to survive at much colder temperatures (Fields, 1992). Different insect species show great differences in susceptibility to cold. *Tribolium castaneum* and *T. confusum* are among the most susceptible to cold while *P. interpunctella* is an example of a relatively cold-tolerant species (Fields, 1992). The gender of the insect can also play significant roles in cold susceptibility; *T. castaneum* females are more susceptible to cold then males when unacclimatized (Edwards, 1958).

Cold temperature exposure has been used commercially to both cease population growth and kill insects in grain (Burks et al., 2000). Grain chillers cool seed usually to 15°C thereby suppressing insect populations. Such chillers are used commercially in Australia, USA and some European countries (Fields and White, 2002). Chilled aeration, in which cold ambient air is circulated through grain by a large fan or fans, is useful in cold climates (or seasonally cold climates like the Prairies). Depending on the temperature, this can control insects in the product and protect the product from future

infestations if used for a long enough time (Burks et al., 2000). Ranalli et al. (2002) report that chilled aeration effectively controls *R. dominica*, *S. oryzae* and *O. surinamensis* in stored rice.

Freezing out mills, by opening up outside doors when temperatures go below 0°C, can help reduce or eliminate pest populations (Posner and Hibbs, 2005). However, it is not practical in flour storage areas because flour is an effective insulator. Flour harbourage sites also need to be removed if the treatment is to be effective, and equipment may need to be protected from freezing (Posner and Hibbs, 2005).

Impact devices

Impact devices are commonly used in flour mills. Once such device is called the entoletor, so-called because of its observed effects on insect populations. It is worth noting that entoletors were invented in the 1940s to reduce particle size during the milling process, not to destroy insects (Plarre and Reichmuth, 2000). Entoleters are centrifugal machines consisting of two spinning discs attached by steel pegs placed on the edges of the discs. There are typically two or more rows of these pegs. The food product is fed into the machine and then is thrown outwards by centrifugal forces, where it hits the spinning pegs and the machine casing (Plarre and Reichmuth, 2000). Death results as a result of physical injury upon hitting the pegs, and/or an inability to molt following impact. More impacts result in greater mortality rates. Entoleters have been shown to have significant effects on different life stages of insects, especially eggs (Plarre and Reichmuth, 2000).

Entoleters are ideal for use in flour mills because of the small particle size of flour and the high mortality rate seen when insects are impacted in flour. Product alteration can be a concern however. In North America entoleters are thought to have little effect on the final product, but in Europe there is debate over this issue (Plarre and Reichmuth, 2000). Throughput is also an issue since these machines can slow down throughput significantly and consequently they may be bypassed during the milling process. Larger machines are necessary in order to make entoleters more cost-effective and effective at controlling insects (Plarre and Reichmuth, 2000).

Pneumatic conveyance is another impact method of insect control. Paliwal et al. (1999) report 98% mortality of adult *T. castaneum* and *C. ferrugineus* when grain of 14% moisture content is pneumatically conveyed at a rate of 5.5 tons/hr. In addition to possible direct control of insects, pneumatic conveyance systems in flour mills help increase cleanliness and sanitation. Such systems also take up comparatively little room so there is space for additional equipment (Cotton, 1958).

Other alternative methods

Parasitoids

There are many predators associated with stored product insects. Some of the predatory insects present in Canada that attack grain insects are in the order Hemiptera and include *Lyctocoris campestris* (F.) (the stack bug) and various *Xylocoris* spp. (cereal bugs). There are many parasitoid wasps as well: Ichneumonidae attack *Ephetia* and *Plodia* spp., *Holepyris sylvanidis* (Brethes) is an external parasitic wasp of *Tribolium* spp., *Holepyris hawaiiensis* (Ashmead) attacks *Plodia* and *Ephetia* spp, *Cephalomonia*

spp. attack various species of beetles (not including *Tribolium* spp.), *Habrobracon* spp. attack various grain moths, and *Trichogramma* spp. attack a wide range of moths (Rees, 2004).

There are advantages to using parasitoids and predators as a control measure. Resistance is not reported and is unlikely to be, since parasitoids and hosts co-evolve (Schöller and Flinn, 2000). Using parasitoids is also effective and simpler to register than chemical insecticides (Fields and White, 2002). Releasing such predators as a control measure can also help to reduce overall insect contamination in the commodity. Augmentative release of the parasitoid wasp *Theocolax elegans* controls *R. dominica* and an overall decrease in the number of insect fragments found in flour is reported (Flinn and Hagstrum, 2001).

There are several practical difficulties with using parasitoids and predators to control insects however. Many of these parasitoids and predators are difficult to obtain commercially. They do not completely suppress problematic insect populations since total annihilation of a host species would result in their own extinction. Predators and parasitoids also need to be released when pest populations are low and are ineffective at controlling large populations like those typically present before a fumigation (Schöller and Flinn, 2000).

Integrated pest management

In integrated pest management, pest populations are monitored and control measures are employed only when "the damage caused by the pests exceeds the cost of pest management" (Subramanyam and Hagstrum, 2000; p. 8). Instead of controlling for

pests on a predetermined schedule (i.e. fumigation twice a year), one controls for pests only when their populations reach the economic threshold, i.e. a predetermined point just before pest populations become large enough to cause economic damage, and a point at which initiating control measures can prevent economic damage from occurring (Pedigo, 1996). There is an emphasis on non-chemical control alternatives in IPM (Subramanyam and Hagstrum, 2000). Sampling and sanitation, discussed below, play crucial roles in IPM practices.

Sampling

In order to determine when pest populations have exceeded economic threshold, i.e. the level at which control measures should be used, it is important to monitor such populations (Subramanyam and Hagstrum, 2000). Visual inspection is a commonly-used monitoring tool, and it is the most important and cost effective part of a sanitation schedule when performed properly (Mills and Pedersen, 1990). In Canadian flour mills, visual counts of *Tribolium* in and around equipment and on the floor are usually recorded once a week (Hawkin, personal observation). Insect tracks are also useful signs of insect presence (Fields and White, 2002).

Traps are also effective monitoring tools. Trap sampling helps to monitor overall populations of insects and helps locate sources of infestation. Such sources can be controlled through sanitation and contact insecticide use before it spreads, necessitating fumigation (Fields and White, 2002; Subramanyam and Hagstrum, 2000). The addition of aggregation pheromone to traps can help increase their efficacy (Javer et al., 1990). Because of this, many monitoring traps contain synthetic aggregation pheromones.

A variety of monitoring traps baited with 4,8-dimethyldecanal, aggregation pheromone of *T. castaneum* and *T. confusum*, have been commercially available for years. Many of these traps are impractical for use in flour mills however due to design flaws. The Plexiglas trap, for example, is not covered and quickly becomes full of filth. Other traps allow insects to escape, or do not have enough entry holes to effectively capture insects (Mullen, 1994). The Dome Trap® (Trécé Inc, Adair, Oklahoma, USA; Mullen et al., 1992) has been used successfully in flour mills. This trap is a pitfall trap baited with both food attractants and aggregation pheromone. Once attracted to the trap, a beetle climbs up an incline and then falls into a pit from which it cannot escape. This trap is easy to use and maintain, it is reusable, durable, covered so it does not fill with flour, and its design does not allow for insects to escape (Mullen et al., 1992). The Dome Trap has been used to monitor insect populations before and after fumigation (Campbell and Arbogast, 2004; Small, 2007) and spatial distribution of insects before and after increased sanitation in pet stores (Nansen et al., 2004).

Monitoring populations can decrease the frequency of fumigation since fumigations can occur when pest populations dictate it to be necessary, rather than on a set calendar schedule (Subramanyam and Hagstrum, 2000). Sampling with traps only gives relative population numbers however, not absolute ones, and many environmental factors, i.e. temperature, lighting, trap placement and the availability of food, can influence the numbers caught in traps (Burkholder, 1985; Toews et al., 2005; Phillips et al., 2000). Trap design, age of the lure and the type of lure used (i.e. laminate, rubber or membrane) can all affect trap catches as well (Hussain et al., 1994b; Phillips, 1997). In order to decrease continued reliance on regularly scheduled fumigations, research into the

relationship between absolute population numbers and the numbers of insects in different types of traps is required.

Pheromone-baited traps have one significant drawback; pheromones are specific to species. Because of this, monitoring with pheromone traps requires knowledge of the pests at issue. Also, since there is often more than one species present (Cox and Collins, 2002; Campbell and Arbogast, 2004), proper monitoring with insect-specific traps can be difficult and laborious.

Food lures are a possible solution to this problem. Food lures, such as bait bags (mesh bags containing various food items), can attract multiple species and are cheaper to make and use than pheromone lure. However, food lures can become sources of infestation themselves because they contain attractive food items and often do not kill or retain insects (Pinniger, 1990). Using food volatiles instead of food to attract insects, and using retaining/killing traps will solve this issue. There has been some research on isolating food volatiles for traps (Collins et al., 2007) but much more research is required.

Sanitation

Sanitation involves the removal and exclusion of insects. The design of a plant affects the amount of harbourage available for insects. Voids of any kind, whether they be in the floor, in walls or around equipment, harbour pests. Concrete floors have fewer cracks than wooden floors and give insects fewer places to hide. In older buildings, cracks in wooden floors can be sealed to decrease refuges for insects (Imholte and Imholte-Tauscher, 1999). Pests can be excluded from food processing facilities through placing screens of at least 16 mesh on windows, using air curtains at entrances to the

facility, rapidly removing trash, avoiding use of baseboard and false ceilings and leaving the grounds surrounding the plant well-drained and clutter-free. Exposed equipment and overhead beams need to be inspected for insects and cleaned on a regular basis (Troller, 1993).

Cleaning disrupts the habitat of insects in addition to physically removing them. It may have other effects on population growth as well. *T. confusum* and *T. castaneum* lay more eggs in deeper flour (Sokoloff, 1972), so less flour results in fewer eggs. Changes in oviposition rates have also been reported when beetles could choose between small amounts of flour (Campbell and Runnion, 2003). Removing excess flour on the floors of flour mills can thereby decrease the growth of *Tribolium* populations in an indirect manner.

Sanitation is primarily a preventative measure of insect control however and it can be labour-intensive and costly. Precision cleaning schedules based upon sampling are necessary for maximum control with minimum expense. Trematerra and Gentile (2006) used extensive sampling to create precision cleaning schedules focusing on localized populations. These schedules resulted in suppressed populations as compared to the populations seen at the same time the previous year, and populations increased once these monthly cleaning schedules were abandoned.

Good sanitation is essential if other methods of insect control are to work at their peak. Fields and White (2002) emphasize the need to remove all food and flour residues prior to a heat treatment, due to their insulating natures. Arthur (1998) reports that contact with flour increased survival rates of *T. castaneum* previously exposed to the contact

insecticide cyfluthrin, either because it directly removes the insecticide from the beetle or because food intake results in detoxification.

Benefits and costs associated with non-chemical methods of control

Non-chemical control methods have many advantages over traditional insecticides. Little, if any, regulatory approval is required while insecticides cost millions of dollars to test and register. Non-chemical control methods also leave few, if any, residues on the commodity, insect resistance is not a concern and the health and environmental effects are usually negligible (Fields and White, 2002). Consumers are also quite accepting of most non-chemical alternatives and wish them to be used in place of conventional methods (Miles and Frewer, 2001).

Primary concerns with non-chemical control methods lie in their efficacy, limited application and speed of action. None of the control methods mentioned above suppress insect populations as broadly and extensively as methyl bromide. Even heat treatments, which have been used successfully in many facilities, are not as effective and usually need to be performed three or four times a year compared with once or twice with methyl bromide (Fields and White, 2002). As well, most non-chemical control methods are slow-acting and there are issues concerning specificity of action, i.e. species-specific parasitoids and pheromone traps.

The most crippling problem with non-chemical methods is cost. The most economically viable non-chemical method outlined is heat treatment; it costs approximately the same as methyl bromide fumigations on a yearly basis, but shutting down a facility three or four times a year results in increased economic losses through

decreased productivity. Cost is a major reason why IPM has not been more widely employed by grain farmers (Adam et al., 2006) and why there is continued reliance of insecticides (Ducom, 2006). Even consumers who are concerned about pesticides and food safety often do not buy organic foods due to the increased expense (Miles and Frewer, 2001).

Summary and rationale for study

Stored product insects infest a wide variety of commodities and controlling them is difficult. The difficulties encountered are due to their biology; stored product insects have high reproductive rates and can survive and breed within a wide range of temperatures and humidities (Rees, 2004). *Tribolium castaneum* and *T. confusum*, two closely related insect pests, are examples of successful and hardy insect pests. In order to suppress their populations in facilities such as flour mills, an understanding of their biology and behaviour is critical. Chapter 5 presents studies on the burrowing tendencies of mill and laboratory strains of *T. confusum* and *T. castaneum*, as well as the movement rates of beetles directly from a Canadian flour mill versus laboratory strain beetles.

Laboratory research on *T. castaneum* and *T. confusum* has been extensive. Sokoloff has detailed much of this research in three volumes, two of which have been cited in this paper. Research into the biology and behaviour of these two beetles in flour mills is needed to supplement this work. Disturbances such as shaking, sieving and rolling occur in flour mills, but it is not known how these types of disturbances may affect the fitness of *T. castaneum* and *T. confusum.* It is also not known how the

availability of harbourage affects the fecundity of these two species. Chapter 3 presents new studies in these areas.

Field research into the behaviour and interactions of these two species is needed as well. Park's competition studies (Park, 1948; 1954; 1957) indicate that these two species cannot coexist, but whether they can infest the same facility is unclear. Chapters 4 and 5 contain data on species mixtures in a Canadian flour mill, both inside equipment and in various easily-accessible areas in the mill.

There are many unanswered questions concerning *Tribolium* pheromone traps. As mentioned previously, the relationship between absolute population size and the numbers caught in traps has been unclear; Chapter 5 presents studies on this issue. It also has not been known whether traps could be placed outside flour mill equipment, to monitor for insect infestation inside the equipment; Chapter 4 contains work on this area.

Both *T. confusum* and *T. castaneum* probably have an aggregation pheromone involving a mixture of 4R, 8R-(-)- and 4R, 8S-(+)-4,8-dimethyldecanal (Suzuki et al., 1984), and pheromone traps on the market contain a mixture of these two isomers (Hussain et al., 1994b; Mullen et al., 1992), but different ratios of the two isomers probably attract both species to different degrees (Suzuki et al., 1984). There are also reports that different strains of *T. castaneum* differ in their attraction to, and production of, aggregation pheromone (Boake, 1984). Research is needed to see if different strains and species are caught in traps at different rates. Chapter 5 presents such research.

There are no widely adopted alternatives to methyl bromide at this time. Ducom (2006) cites the positive qualities of methyl bromide as reasons for this:

> "As MeBr was so effective on a very broad spectrum of pests and so cheap, recognized all over the world as such, it has inhibited a lot of research. The parties of the Montreal Protocol were surprised to find that alternatives is so difficult [*sic*]. The research really began after its inclusion in the ozone depletion substances in 1992." (Ducom, 2006; p. 511).

If non-chemical control measures are to be more widely employed, they must become economically competitive. IPM strategies are promising in this regard, because IPM is concerned with balancing pest control costs and losses due to pests. As mentioned earlier, sampling is an integral part of IPM, and sampling requires an understanding of the biology of pests. This thesis provides new information on the behaviour of different strains of *T. confusum* and *T. castaneum,* as well as on the efficacy of pheromone trap monitoring for different strains and species of these beetles. It is hoped that such information will lead to a more thorough understanding of *T. confusum* and *T. castaneum* in flour mills, more effective traps and better methods of sampling that provide reliable predictions of pest populations.

Chapter 3: The effect of disturbance and harbourage on the fitness of *Tribolium castaneum* and *Tribolium confusum*

Abstract

Tribolium confusum Jacquelin du Val and *Tribolium castaneum* (Herbst) were subjected to 24-hour sieving, shaking and rolling disturbances in the laboratory to simulate those seen in flour mill equipment. After disturbance, fecundity and dispersal were measured. *Tribolium confusum* fecundity was not affected by disturbance, and sieving decreased *T. castaneum* fecundity. Sieved beetles dispersed slower than unsieved beetles in both species, rolling reduced *T. confusum* dispersal rates, and shaking reduced *T. castaneum* dispersal rates. In another experiment, beetles (all undisturbed) were given differing amounts of flour (0, 0.5 g, 2 g, 25 g), with or without harbourage. All beetles laid more eggs in larger amounts of flour, and harbourage only affected egg laying in *T. castaneum* and only at the 2 g flour level. For all fecundity experiments, *T. castaneum* laid more eggs than *T. confusum*.

Introduction

There is a great need for alternative methods of stored product insect control in flour mills. The use of insecticides has been greatly reduced over the last decade. The most commonly-used fumigant, methyl bromide, was officially banned in developed countries as of 1 January 2005 and today can only be used through critical use exemptions (Technology and Economic Assessment Panel, 2004; Technology and Economic Assessment Panel and Methyl Bromide Technical Options Committee, 2006).

Consumers have also become more wary of chemical pest control in general and demand alternatives (Fields et al., 2001; Mittelstaedt, 1991). Food must still be free of insects and insect parts however. Through an understanding of the insect pest's biology, one can develop practical and alternative control strategies.

Insect pests require harbourage and food. Oviposition rates for *T. confusum* and *T. castaneum*, two common insect pests found in Canadian flour mills, are affected by factors such as temperature, humidity, availability of food and population densities (Sokoloff, 1974). Because of this, sanitation can significantly impact insect populations but sanitation programs can be expensive and time-consuming. An increased understanding of the effects that harbourage and disturbance inside food processing equipment have on *T. confusum* and *T. castaneum* could lead to more targeted, effective and economical cleaning programs.

This study explored the effects of disturbance, harbourage and food availability on the fitness of *T. castaneum* and *T. confusum*. Disturbance effects on fecundity and dispersal were measured, along with the effects of harbourage and food availability on fecundity. The disturbances used (shaking, rolling and sieving) simulated those found inside food processing equipment in flour mills.

Methods

There were three preliminary experiments. The first experiment examined the effects of disturbance on the fecundity of *T. castaneum* and *T. confusum*. Three types of disturbance were used; sieving, rolling and shaking. Unbleached white flour is hereafter referred to as plain flour, and unbleached white flour + 5% brewer's yeast is hereafter referred to as flour.

For the sieving treatment, beetles were placed on 425 µm sieves (4 sieves, one sex/species per sieve), the sieves were put into an automatic shaker (two sieves on each side, stacked on top of each other) and the shaker was left on a high setting for 24 hours (220 motions/minute; Figure 3.1). For the shaking treatment, beetles were placed into experimental vials (three virgin females and three virgin males per vial), the vials were attached to the same automatic shaker and the shaker was set on high for 24 hours (Figure 3.1). In the rolling treatment, beetles were placed in experiment vials (three virgin females and three virgin males per vial), the vials were placed into a 2 L empty jar and the jar was rolled on a roller stand for 24 hours (96 rpm; Figure 3.2).

There were two control groups for this experiment. The controls for the sieved beetles were placed on 150 µm sieves next to the automatic shaker during the disturbance period, and controls for the rolled and shaken beetles were left on the laboratory counter. The mean temperature and relative humidity on the counter ± SEM for this experiment was 18.5 ± 0.07°C (range: 17.5-22.5°C), 23.7 ± 0.05% RH (range: 23.4-27.2%), as measured by HOBO® data loggers (Onset Co., Bourne MA).

Figure 3.1. Setup for shaking and sieving disturbances. For the shaking condition, beetles were placed in each vial and vials were attached to an automatic shaker. For the sieving condition, beetles were placed on 425 µm sieves which were put in the automatic shaker. Undisturbed beetles for the sieving condition were placed on 150 µm sieves.

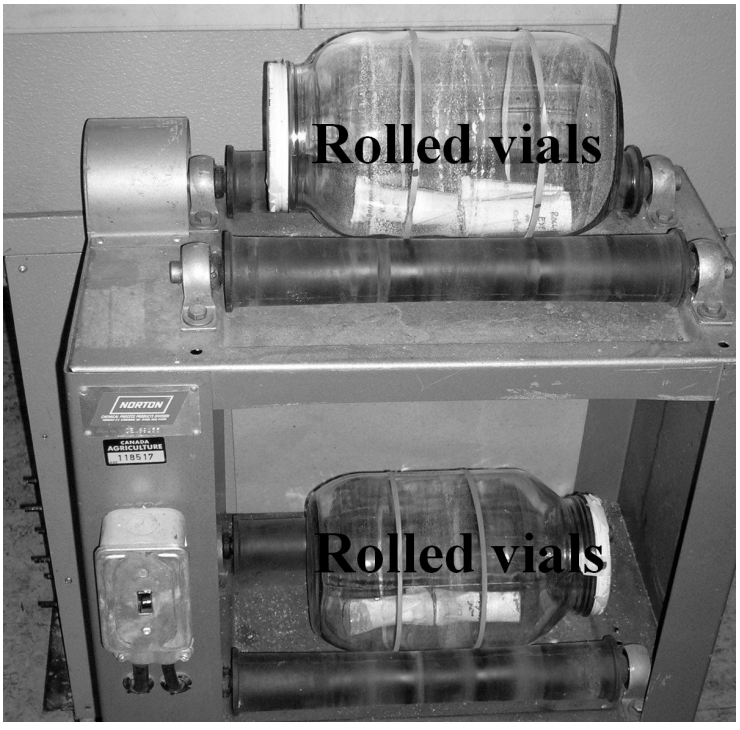

Figure 3.2. Setup of the rolling disturbance. Beetles were placed in vials containing flour, the vials were placed inside 2 L jars and the jars were rolled on a rolling stand.

After the disturbance, all beetles were transferred to vials containing flour presifted with a 150 μm sieve. Three males and three females were present in each vial. All vials were put in an incubator (30°C, 60% RH) for three days. The number of dead adults was then noted, the eggs were sifted out using a 180 μm sieve and the eggs were counted.

The second experiment examined the effects of disturbance on dispersion of *T. confusum* and *T. castaneum*. To measure dispersion, dispersal tubes were used (Figure 3.3). The setup of the dispersal tubes was taken from Prus (1963): two vials were connected by plastic tubing and a thread ran between the two vials. The thread acted as a "bridge", allowing the beetles to travel from one vial to the other. The beetles were initially placed in vial A, which contained 8 grams of plain flour. The second vial, vial B, contained no flour. When the beetles traveled to vial B, they fell into the vial and could not escape. The number of beetles in vial B was used as the measurement of dispersion.

Prior to the dispersal experiment, all beetles were mated; males were marked with a small dot of Wite-Out® (BIC USA, Shelton, Connecticut) and males and females were placed together in flour and left in the incubator for a week prior to the experiment. Beetles were mated to better simulate mill conditions (beetles in mills are probably mated soon after eclosion). After mating, beetles were subjected to sieving, rolling or shaking treatments for 24 hours and undisturbed beetles were used for comparison purposes. In the rolling and shaking conditions, 5 males + 5 females were placed in vials, 3 vials/treatment and then beetles were disturbed as described previously. In the sieving condition, there were two sieves used (one sieve/species, males and females from the

same species combined on the same sieve), one sieve was placed on each side of the automatic shaker and then beetles were disturbed the same as before.

After disturbance, beetles were transferred into dispersal tubes (5 mated males and females per tube.) The number of beetles in vial B was measured for 88 hours; measurements were taken once an hour for hours 1-5, 18-20, 24-26, 28, 47, 49, 51, 67 and 88 after beetles were placed in the tubes. The mean temperature and relative humidity during this experiment ± SEM was 20.1 ± 0.10°C (range: 19.0-24.0°C), 26.5 ± 0.1% RH (range: 23.6-29.3%), as measured by data loggers.

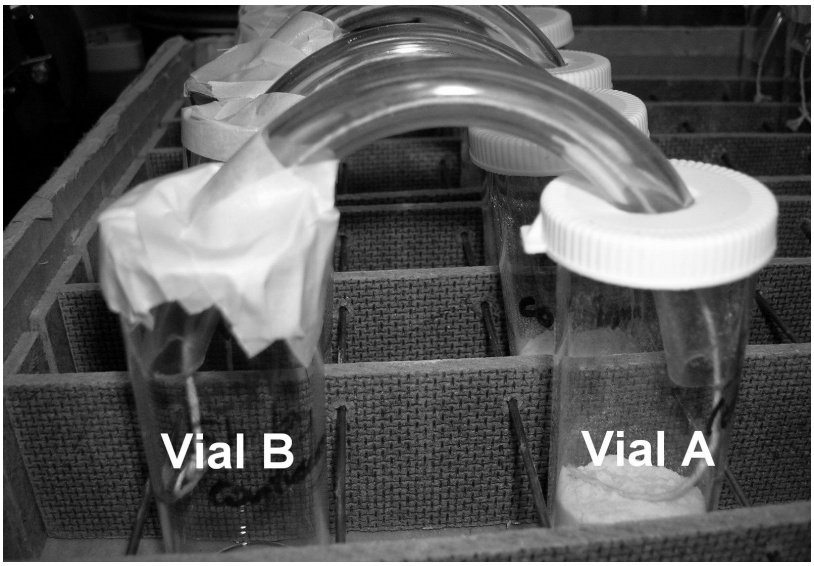

Figure 3.3. Dispersal biossay. Two vials were connected by plastic tubing and a thread ran between the two vials. The right vial (vial A) is the vial into which beetles were initially placed. Beetles traveled to vial B by climbing up the thread. When the beetles reached vial B, they fell into the vial and could not escape. (Setup taken from Prus, 1963.)

For the third and final experiment, the effects of harbourage and food availability on fecundity were measured. Treatments were set up in petri dishes, each petri dish

containing 5 virgin males and 5 virgin females (Figure 3.4). In four of these treatments, corrugated cardboard (harbourage) was present; in the other four treatments there was no cardboard (no harbourage). Different amounts of flour (0 g, 0.5 g, 2 g and 25 g) were used in both harbourage and non-harbourage conditions to test the effects of food availability, making a total of 8 treatments (4 different amounts of flour × harbourage/no harbourage). Once the beetles were in the petri dishes, the dishes were placed in the incubator for three days (30°C, 60% RH). After three days, dead adults were noted, a 180 µm sieve was used to retrieve the eggs, and the eggs were counted.

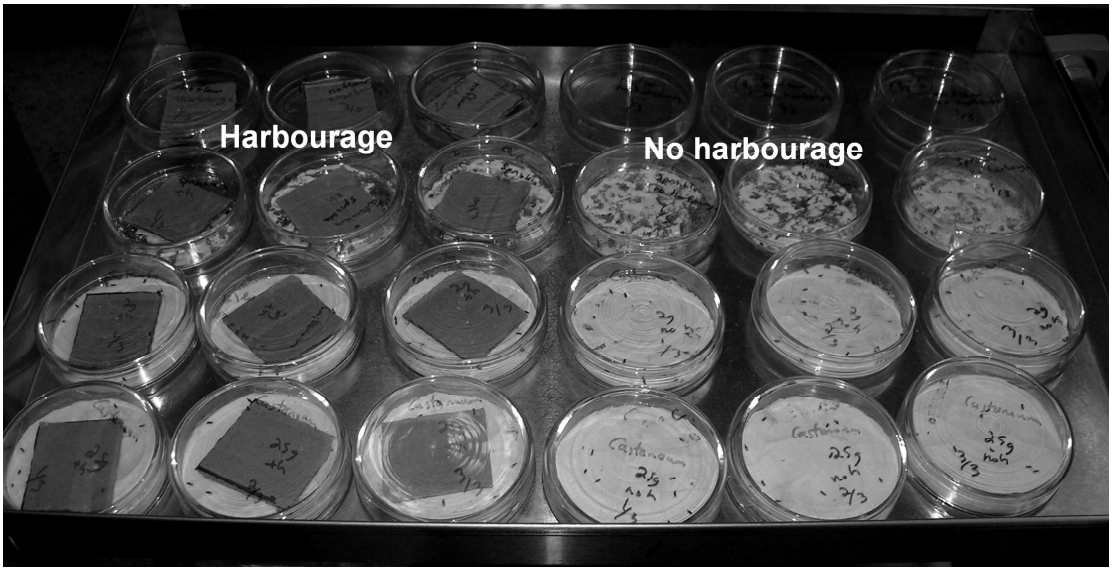

Figure 3.4: Setup of harbourage versus fecundity experiment. Beetles were placed in petri dishes and the number of eggs laid after three days was counted. The left half of the petri dishes contain harbourage and the right half contain no harbourage. Differing amounts of flour were used within harbourage or no harbourage conditions; see text.

For the three experiments, there were three repetitions/treatment in most cases. For disturbance versus fecundity, there were four repetitions of unsieved *T. confusum* controls used in analysis, and four repetitions of sieved and unsieved *T. castaneum* used.

Twelve beetles of each species and sex were placed in a sieve for the disturbance period (4 sieves, 1 sieve/species/sex) and beetles were divided into four repetitions after disturbance (3 males + 3 females/repetition). For disturbance versus dispersal, two repetitions were used in analysis for the sieving condition for both species. Twenty four beetles from each species (12 males + 12 females) were placed in a sieve for the disturbance period (2 sieves, 1 sieve/species) and beetles were divided into two repetitions after the disturbance (5 males + 5 females/repetition). (Four extra beetles were placed in each sieve in case there was mortality during the disturbance.) Statistical analysis for all experiments was performed using SigmaStat® (Systat Software Inc., San Jose, California).

Results

In the first experiment, disturbance versus fecundity (Table 3.1), the sieving treatment for both species was repeated due to high mortality. A shaker accident during the first trial resulted in 100% mortality for *T. castaneum*. During the second attempt, mortality was still too high for statistical analysis for *T. castaneum*. The third repetition for *T. castaneum* and the second repetition for *T. confusum* were used in analysis. Some of this variance in mortality was due to a problem with the experimental set-up; follow-up work indicated that mortality rates were greater in the left-hand side of the automatic shaker than the right-hand side, so mortality rates changed depending on what side of the sifter the beetles were placed. Egg counts were converted to mean number of eggs per female and all females that survived the disturbance were used to calculate number of

eggs per female, including those who died between the disturbance and the retrieval of eggs.

Sieving significantly affected fecundity in *T. castaneum*, with sieved beetles laying fewer eggs than unsieved (Mann-Whitney Rank Sum Test used due to failed normality, data not transformed; U=10, p=0.03). For *T. confusum*, sieving did not have a significant effect on fecundity (Mann-Whitney Rank Sum Test due to failed equal variance, data not transformed, U=10, p=0.6). The other disturbances did not affect fecundity (rolling: *T. confusum*: Mann-Whitney Rank Sum Test used due to failed equal variance, data not transformed, U =6, p=0.67; *T. castaneum*: t=-2.4, p=0.08; shaking: *T. confusum*: t=0.13, p=0.90; *T. castaneum*: t=0.6, p=0.60). *Tribolium castaneum* laid significantly more eggs than *T. confusum* across disturbance conditions (Mann-Whitney Rank Sum Test due to failed normality, data not transformed, U=285, p<0.001).

Table 3.1. The effects of disturbance on the fecundity of *Tribolium confusum* and *Tribolium castaneum*. Beetles were subjected to a 24-hour disturbance (either sieving, rolling or shaking) and the number of eggs laid in the following three days was counted. Mean number of eggs/female ± SEM are presented. There were three repetitions of each treatment unless otherwise noted.

Insect	Disturbance type	Mortality (%)		Fecundity (mean # of eggs/female) ± SEM		
		Undisturbed	During disturbance	During egg-laying	Undisturbed	Disturbed
T. confusum	Sieving[1]	0	9[3]	6 ± 6	29 ± 1	20 ± 9
**	Rolling	0	0 ± 0	11 ± 11	33 ± 10	27 ± 6
	Shaking	0	0 ± 0	0 ± 0	26 ± 2	25 ± 3
T. castaneum	Sieving[2]	0	0[3]	29 ± 8	50 ± 4*	0.6 ± 0.2*
**	Rolling	0	0 ± 0	0 ± 0	46 ± 2	53 ± 1
	Shaking	0	6 ± 6	6 ± 6	50 ± 3	37 ± 17

1. There were four repetitions of control treatment.
2. There were four repetitions of control and experimental treatment.
3. All beetles of a particular species were placed in the same sieve during disturbance, so no SEM is given.

* Sieved versus undisturbed *T. castaneum* - U=10, p=0.03

** Significant fecundity differences between the two species overall - U=285, $p<0.001$

In the second experiment, disturbance versus dispersal (Table 3.2), the "mean time to disperse" was calculated for each disturbance treatment. Kaplan-Meier survival analysis (Log-Rank) was performed on dispersion data to determine effects within disturbance treatments; beetles that did not move to vial B by the end of the 88-hour period were censored in the analysis. Sieving significantly affected dispersion for both *T. confusum* (S=30, p<0.001) and *T. castaneum* (S=10, p=0.03), with the sieved beetles moving less than unsieved beetles in both cases. In the case of *T. castaneum*, 18 out of 19 beetles in the sieving treatment did not disperse. Rolling caused *T. castaneum* beetles to disperse slower (S=10, p=0.002), while shaking caused *T. confusum* to disperse slower (S=5, p=0.03).

For harbourage versus fecundity, two and three-way ANOVAs were performed to test for effects of harbourage, effects of flour quantity and differences between species. Fecundity was significantly impacted by the amount of flour present for both *T. confusum* (F=116, p<0.01) and *T. castaneum* (normality failed, F=186, p<0.01). In both species, the most eggs were laid with 25 g of flour and the least amount of eggs with no flour (Table 3.3). Harbourage increased *T. castaneum* fecundity (normality failed, F=11, p=0.005). In particular, harbourage positively affected fecundity in the 2 g harbourage/no harbourage situation (t=5, p<0.001). For *T. confusum*, harbourage did not affect fecundity. *Tribolium castaneum* laid significantly more eggs that *T. confusum* across treatment conditions (three-way ANOVA, F=126, p<0.001). There were significant interactions between species, amount of flour and harbourage/no harbourage (F=2.998, p=0.05), mostly because harbourage affected fecundity at only the 2 g level of flour with only one species (*T. castaneum*).

Table 3.2. The effects of disturbance on the dispersal of *Tribolium confusum* and *Tribolium castaneum*. Beetles were subjected to a 24-hour disturbance (either sieving, rolling or shaking) then placed in dispersal tubes (Figure 3.3) and the time to disperse from the initial vial was measured. Mean time to disperse ± SEM is presented. There were three repetitions of each treatment unless otherwise noted.

Insect	Disturbance type	Mortality (%)			Mean time to disperse (h) ± SEM	
		Undisturbed	During disturbance	During dispersal	Undisturbed	Disturbed
T. confusum	Sieving[1]	0 ± 0	0 ± 0	0 ± 0	8 ± 5*	70 ± 7*
	Rolling	0 ± 0	1 ± 1	0 ± 0	11 ± 4	22 ± 7
	Shaking	0 ± 0	0 ± 0	1 ± 1	3 ± 1**	15 ± 6**
T. castaneum	Sieving[1]	0 ± 0	0 ± 0	1 ± 1	51 ± 10[2]***	85[3]***
	Rolling[4]	0 ± 0	0 ± 0	0 ± 0	31 ± 7****	62 ± 6****
	Shaking[4]	0 ± 0	0 ± 0	0 ± 0	31 ± 7	47 ± 7

1. There were two repetitions of the sieving experimental treatment.
2. One beetle escaped during the dispersal period.
3. Only one beetle dispersed from the initial vial within 88 hours.
4. The undisturbed group was the same for rolled and shaken conditions.

* Sieved versus undisturbed sieved *T. confusum: S* (Kaplan-Meier Survival Analysis) =30, $p<0.001$

** Shaken versus undisturbed *T. confusum: S*=5, p=0.03

*** Sieved versus undisturbed *T. castaneum: S*=10, p=0.002

**** Rolled versus undisturbed *T. castaneum: S*=10, p=0.002

Table 3.3. The effect of harbourage on fecundity of *Tribolium confusum* and *Tribolium castaneum*. Beetles were placed in petri dishes with or without harbourage and given differing amounts of flour. The number of eggs laid in the subsequent three days was counted. There were three repetitions of each treatment unless otherwise noted.

Insect	Amount of flour (g)	Mortality (% of beetles)		Fecundity (mean # of eggs/female ± SEM)			
		Harbourage	No harbourage	Harbourage	No harbourage	Harbourage vs. no harbourage +	Differences between flour amounts ++
T. confusum	0	0	3	0 ± 0	0 ± 0[1]	ns	a
	0.5	0	0	3 ± 1	1 ± 1	ns	ab
	2	0	0	6 ± 2	5 ± 1	ns	b
	25	0	0	25 ± 2	24 ± 2	ns	c
T. castaneum	0	0	0	0 ± 0	0 ± 0	ns	a
	0.5	0	0	11 ± 3	11 ± 2	ns	b
	2	0	0	23 ± 1	11 ± 2	*	c
	25	0	0	42 ± 1	38 ± 2	ns	d

1. One repetition of this treatment was excluded from analysis.

ns = not significant

+ Overall effect of harbourage significant for *T. castaneum* fecundity- $F=11$, $p=0.005$
++ Overall effect of flour significant for *T. confusum* fecundity ($F=116$, $p<0.001$) and *T. castaneum* fecundity ($F=186$, $p<0.001$). Different letters indicate significant differences between amounts of flour.

* harbourage/no harbourage - $t=5$, $p<0.001$

Discussion

The sieving disturbance had the greatest effect on beetles. It was the only disturbance that caused any change in fecundity (seen with *T. castaneum*), and it resulted in slower dispersal rates for both species. The shaking speed seen in this study (220 motions/min) corresponds to rates seen in mill shakers (110-350 motions/min; Posner and Hibbs, 2005), so it is possible that beetles in mill sifters experience similar effects to those seen here. Beetles in mills go through many sieves however, since sieves are stacked on top of each other in flour mill equipment. The variance in mortality rates for the sieving disturbance is problematic and much of this can be attributed to the experimental setup (see results). The high mortality seen with sieved *T. castaneum* during egg-laying is also an issue, since it is not clear when these beetles died and if females had a chance to lay any eggs before death. While it does appear that sieving affects the beetles' fecundity and dispersal rates, further research into the effects of sieving should be done before reaching any conclusions.

Rolling did not affect oviposition rates in this study, but it did affect the rate of dispersal for *T. castaneum*. The rate of rolling was lower in this study than rolling seen in side rollstands in mills (96 rpm versus 500-800 rpm; Posner and Hibbs, 2005). It is possible that a more forceful disturbance, at the rpm rates seen in mills, may have effects on beetles not seen here. Beetles live and breed in rollstands (i.e. machines used to grind flour in mills; Chapter 4), so further studies on the effects of rolling disturbances could be of practical use in flour mills.

Shaking did not affect oviposition rates in this study, contrary to the findings of Rich (1969). *Tribolium castaneum* in Rich's (1969) study were shaken with an electrical

vibrator for 10 seconds every 2 hours, and oviposition rates were measured every 4 hours. There were higher oviposition rates at 12 hours than at 4, even though there had been more disturbances by 12 hours. This indicated that beetles adapted to the disturbance. In this study, beetles were disturbed constantly for 24 hours and oviposition rates were measured once, over 3 days after the disturbance. This increase in disturbance and prolonged recovery time may have given the beetles time to adjust to the disturbance and even compensate for effects experienced early on in the experiment.

Tribolium confusum dispersed quickly in this experiment, with the majority of these beetles moving over to vial B in the first hour. *Tribolium castaneum* beetles, on the other hand, took many hours to disperse in both this experiment and in Prus (1963). This species difference in dispersal rates may exist because of opposing flour preferences. *Tribolium castaneum* prefers fresh flour to conditioned flour, i.e. flour that has been lived in by *Tribolium* (Sokoloff, 1974; Ghent, 1963) but *T. confusum* is repelled by fresh flour, dispersing less from the surface of flour as it becomes conditioned (Sokoloff, 1974; Ogden, 1970; Ghent, 1963). In many of these studies, flour was conditioned by rearing high densities of *Tribolium* on the same flour for months. Flour in a flour mill can be considered "fresh" since it rarely undergoes that degree of conditioning. Since *T. castaneum* burrows into fresh flour, it may be harder to visually detect populations of *T. castaneum* in a mill than *T. confusum*. Increased overall sanitation practices may be more beneficial in mills infested with *T. castaneum* than in mills infested with *T. confusum* because they will help prevent an unforseen population "explosion".

When beetles were left undisturbed in differing amounts of flour, both *T. confusum* and *T. castaneum* laid the most eggs in the largest amount of flour and did not

lay any eggs when given no flour. These results correspond to results from other studies (Sokoloff, 1972) and show that there are fewer beetle eggs laid and decreased populations when there is less food available to the beetles. The finding that *T. castaneum* lays more eggs than *T. confusum* is also consistent with the literature; Park and Davis (1945) report that *T. castaneum* oviposits at a higher rate than *T. confusum* when reared at 29°C and 60-75% relative humidity, conditions similar to those in this study. *Tribolium confusum* was not affected by the presence/absence of harbourage but *T. castaneum* was, laying more eggs when harbourage was present. Much of this difference occurred at one level of flour however (2 g). Because the effect of harbourage is not consistent across flour amounts, it is difficult to extrapolate these results to a flour mill setting.

Tribolium castaneum was the only species whose fecundity was affected by disturbance. *Tribolium castaneum* has previously been reported as more sensitive to environmental conditions than *T. confusum* (Park, 1954; Park, 1955). This species also showed large changes in oviposition rates in the habourage experiment; there were significantly more eggs laid in 25g of flour/2 g/0.5 g/0 g. The harbourage results correspond with Campbell and Runnion (2003), who report that *T. castaneum* can maximize oviposition rates when presented with different patch-sizes of flour. These findings suggest that sanitation in flour mills infested with *T. castaneum* may be more effective at controlling beetle populations than sanitation practices in mills with *T. confusum*. The direct effects of increased sanitation on *T. castaneum* versus *T. confusum* populations in flour mills need to be investigated further.

Chapter 4: Sampling flour beetles in Simons rollstands

Abstract

Rollstands in three Canadian flour mills were internally sampled and the number of adult and pupae *Tribolium* spp. present were counted. The number of adult beetles inside the machines was compared to beetle activity seen outside the machines, which was accessed by counting the number of tracks in front of the machines and the number of beetles caught in monitoring traps placed at the foot of each rollstand. Beetle activity outside the rollstands was not correlated with beetle populations inside the machines. The number of adults inside the rollstands was highly correlated with the number of pupae present, indicating long-term infestation. Adult beetles in the rollstands of one mill were analysed to species (either *T. confusum* or *T. castaneum*). Both species were present but they were spatially segregated from each other. *Tribolium castaneum* was the predominant species.

Introduction

Monitoring pest populations is an integral part of any integrated pest management (IPM) program (Subramanyam and Hagstrum, 2000). Many of the studies concerning population monitoring of *T. castaneum* and *T. confusum*, two important pests in flour mills, focus on monitoring populations using pheromone traps (Campbell and Arbogast, 2004; Small, 2007). However, there are many questions concerning trap monitoring; the relationship between the numbers caught in traps and the absolute size of a population is poorly understood (Phillips et al., 2000), as is the usefulness of pheromone traps to monitor populations inside machinery. There are also no scientific studies on the

usefulness of other sampling methods (i.e. visual counts of dead/live beetles and the number of beetle tracks present) in monitoring beetle populations in flour mills.

This study is concerned with understanding population dynamics of flour beetles (*Tribolium* species) living within rollstands in flour mills. Rollstands are machines used to grind flour in flour mills; Figure 4.1. These machines take significant time to clean and they cannot be cleaned safely unless machines are off. Because of this, frequent in-depth cleaning of all the rollstands in a flour mill is impractical. If pheromone traps and other quick sampling methods could be used to determine which rollstands are heavily infested with flour beetles, more efficient and cost-saving sanitation schedules could be devised.

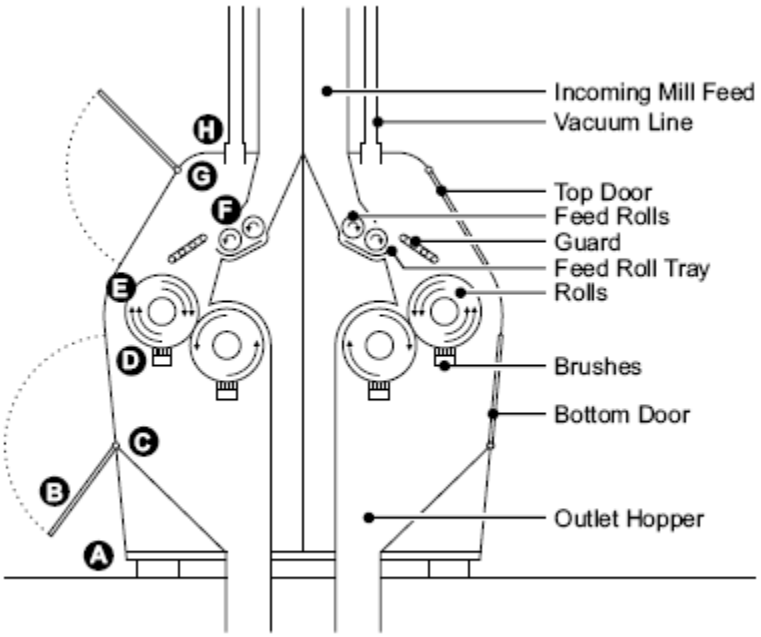

Figure 4.1. Schematic diagram of a Simons rollstand in a flour mill.

The first objective of this study was to compare the beetle populations within rollstands to the amount of beetle activity seen outside the rollstand. Rollstands were sampled in several internal areas known to harbour flour; the number of beetles in the

flour samples was compared to the numbers of beetles caught in pheromone traps placed next to sampled rollstands, along with the number of beetle tracks in front of the sampled rollstand. The second objective was to determine the size of flour beetle populations present within different locations of Simons rollstands. If the relationship between trap counts and population sizes is weak, the second aim would reveal the best way of sampling the Simons rollstand to determine the abundance of flour beetles.

Methods

In three Canadian flour mills, 15-20 Simons rollstands were selected in consultation with mill staff in an attempt to sample both heavily infested and less-infested rollstands. (Simons rollstands were selected because they are particularily prone to infestation.) Rollstands were sampled during the 8-hour shutdown period for the mill. Dome® traps (Trece Inc., Adair, Oklahoma) were placed at the feet of each Simons rollstand to be sampled 2-3 days before sampling; rollstands were in operation between the time traps were put down and sampling began. There was one trap for each rollstand, placed in front of the rollstand at the right-hand side. There were also 21 traps placed in various areas of the mill floor so that spatial analysis of beetle populations beyond the sampled rollstands could be determined.

The Simons rollstands were sampled in eight areas (Figure 4.1, Table 4.1). All flour from the sample areas was vacuumed-up using a custom vacuum (Figure 4.2) and sifted for adult beetles and pupae using a 850 µm sieve. Flour samples were returned to the laboratory and held at 15-20°C for up to 20 days before beetles and pupae were counted. Tracks were counted in front of each rollstand prior to sampling.

Table 4.1. Description of sample areas within rollstands. Each area was vacuumed and the flour gathered was sifted for adult *Tribolium* and pupae.

Sample letter	Corresponding sample area
A	In front of the rollstand
B	Inside surface of bottom door
C	"Dead spots" on outlet hopper, within 19 cm surface of bottom door corner
D	Guards, brushes, panels and bolts
E	"Dead spot" under the top door
F	Area between feed rolls scraped three times, back and forth +contents of feed roll tray (if present)
G	"Dead spots" in upperhand corner of rollstand
H	Right-hand suction port on top of rollstand (incoming vacuum line)

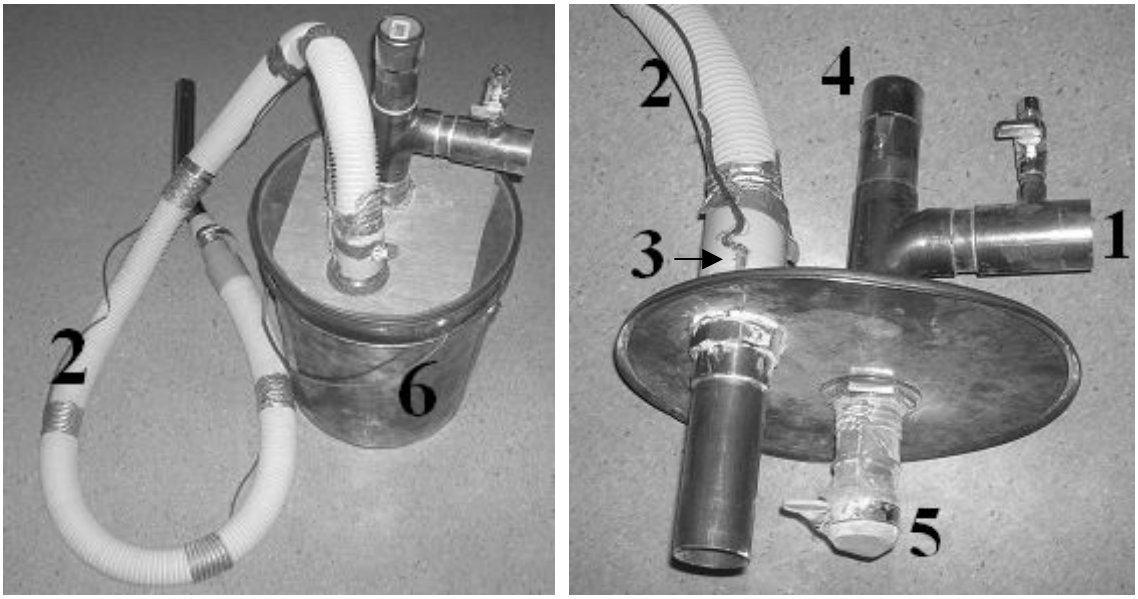

Figure 4.2. Custom vacuum for gathering flour from rollstands. 1. Attachment to mill vacuum system; 2. Hose for vacuuming flour; 3. Ground wire to prevent sparking from static buildup; 4. Adjustable opening to reduce vacuum suction; 5. Mesh screening to prevent loss of large larvae, pupae, and adults; 6. Pail for sample collection.

The first area sampled, A, was the floor area in the direct vicinity of the rollstand. The next area sampled, B, was the inside surface of the bottom door of the rollstand. Sample C consisted of the "dead spots" on the outlet hopper (the area within 19 cm surface of the bottom door). The guards, brushes and adjoining panels and bolts were sample D. The top door was then opened and sample E was collected from the "dead spot" under the door. Sample F consisted of all the flour gathered from scraping, with a long curved metal flong, between the feed rolls three times, in addition to any flour collected in the feed roll tray (if present). Guards in the rollstand were removed to allow this sampling. Sample G was taken from the "dead spots" in the upper inside corners of the rollstand. The final sample, H, came from the right-hand suction port (where the incoming vacuum line is) on top of the rollstand.

This experiment was performed in three different Canadian flour mills (Mills 1-3). In Mills 1 and 2, 20 rollstands were sampled; in Mill 2, 18 were sampled. The number of pupae, adults, tracks found in front of rollstands and beetles caught in the traps were compared using SigmaStat® (Systat Software Inc., San Jose, California). Pupae counts were omitted altogether for Mill 2 because many samples were missing; Mill 2 was the first trial and I started counting pupae late into the sample analysis. Because Mill 1 was known to have both *T. confusum* and *T. castaneum*, all adult beetles collected were identified to species.

Results

The average number of adult beetles and pupae found in the rollstand varied depending on the flour milling stage. Flour milling can be divided into five sequential stages: breaks, sizings, middlings, low grades and tailings. During each stage, flour becomes more finely ground and processed (Posner and Hibbs, 2005). Rollstands in the middlings stage harboured the largest number of beetles (one-way ANOVA, log x+1 transformation, $F=4.8$, $p=0.01$; Table 4.2 and 4.3). The areas inside the rollstand with the least insects were the right-hand suction port and the guards+ brushes + panels + bolts area; both these areas only contained 1-6% of the total number of beetles inside the rollstands. The two most heavily infested areas of the rollstands were the feed roll area (22-82% of beetles) and the "dead spot" on the outlet hopper (11-30%; Figure 4.3). The number of adults was highly correlated with the number of pupae in the samples, both for Mill 1, Mill 3 and overall (Mill 1: $r^2=0.91$, $p<0.001$, $n=127$; Mill 3: $r^2=0.69$, $p<0.001$, $n=120$; overall: $r^2=0.94$, $p<0.001$, $n=247$; Figure 4.4).

Table 4.2. The average number of adult and pupae *Tribolium* spp. in rollstands in three Canadian mills, broken into specific milling stages. Values given are mean ± SEM. Numbers in brackets is the sample size.

Milling stage	Substage	Mill 1		Mill 2		Mill 3	
		Average # of adults/rollstand	Average # of pupae/rollstand	Average # of adults/rollstand	Average # of pupae/rollstand	Average # of adults/rollstand	Average # of pupae/rollstand
Breaks	2nd	0.5 ± 0.5 (4)	0.3 ± 0.3 (4)	27 ± 1 (2)	--	103 ± 48 (4)	11 ± 7 (4)
	3rd	3 ± 1 (6)	1 ± 1 (6)	64 ± 19 (4)	--	93 ± 76 (2)	6 ± 3 (2)
	4th	3 (1)	0 (1)	31 (1)	--	19 ± 10 (3)	3 ± 2 (3)
Sizings	1st	--	--	26 (1)	--	--	--
	Fine sizings	--	--	13 (1)	--	--	--
Middlings	1st	114 ± 64 (3)	31 ± 16 (3)	31 ± 17 (4)	--	300 ± 151 (3)	127 ± 66 (3)
	2nd	1516 ± 495 (2)	1011 ± 164 (2)	--	--	201 ± 15 (2)	101 ± 65 (2)
	3rd	26 ± 20 (2)	1 ± 1 (2)	11 (1)	--	34 ± 10 (2)	33 ± 28 (2)
	6th	--	--	41 (1)	--	--	--
Low Grades and Tailings	1st Low grades	--	--	18 (1)	--	--	--
	1st Tailings	--	--	38 ± 7 (2)	--	--	--
	2nd Tailings	--	--	54 (1)	--	--	--

Table 4.3. The average number of adult and pupae *Tribolium* spp.in rollstands in three Canadian mills according to general mill stages. Values given are mean ± SEM. Numbers in brackets is the sample size.

Milling stage	Mill 1		Mill 2		Mill 3	
	Average # of adults/rollstand	Average # of pupae/rollstand	Average # of adults/rollstand	Average # of pupae/rollstand	Average # of adults/rollstand	Average # of pupae/rollstand
Breaks	2 ± 1 (11)	0.5 ± 0.4 (11)	49 ± 12 (7)	--	73 ± 27 (9)	7 ± 3
Sizings	--	--	20 ± 7 (2)	--	--	--
Middlings	489 ± 288 (7)	302 ± 187 (7)	29 ± 12 (6)	--	196 ± 73 (7)	93 ± 33
Low Grades and Tailings	--	--	37 ± 8 (4)	--	--	--

Table 4.4. The percentage of *Tribolium* beetles found in different areas of rollstands in three Canadian flour mills.

Mill	Beetles (%)							
	Bottom door (B)	Dead spots on outlet hopper (C)	Guards, brushes, panels and bolts (D)	Dead spot under the top door (E)	Area between feed rolls (F)	Dead spots in upper inside corner of rollstand (G)	Right-hand suction port on top of rollstand (H)	n
1	2	11	2	1	82	2	1	20
2	2	30	6	13	22	13	6	19
3	17	26	1	2	29	5	4	15

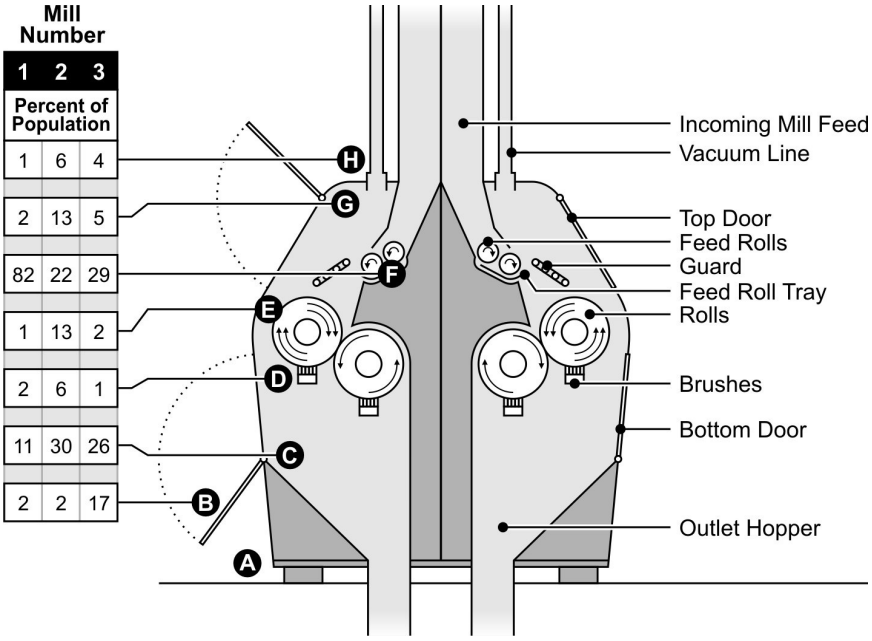

Figure 4.3. A diagram of the percentage of *Tribolium* beetles (by mill) found in different areas of rollstands in three Canadian flour mills.

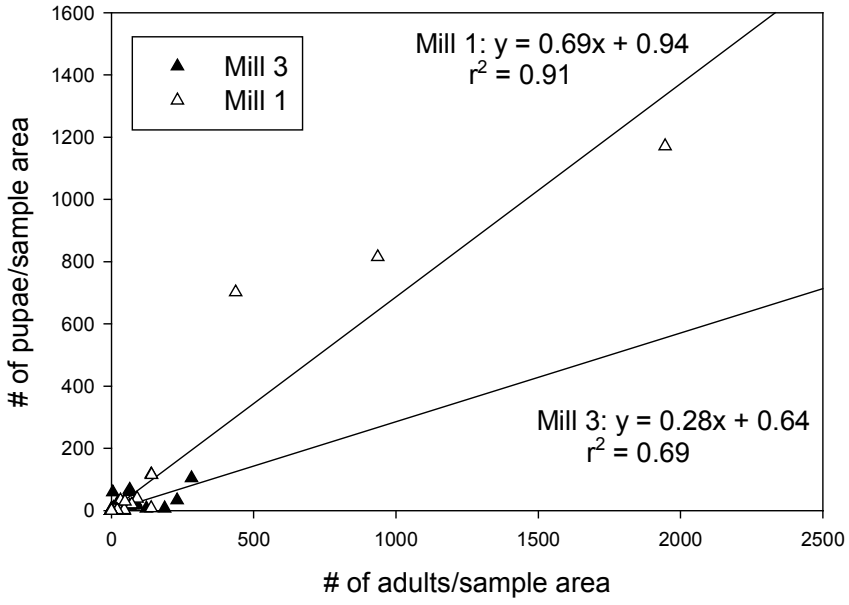

Figure 4.4. Correlation between the number of adult beetles and pupae found in rollstands in two Canadian mills. See text for statistics.

There were few beetles caught in the traps. There were no beetles caught in any of the traps next to the rollstands in Mill 1 the day before and day of sampling and only 3 beetles were caught at Mill 2 (Figure 4.5). The small numbers caught in the additional traps placed in various areas across the floor made spatial analysis of beetle populations impossible. The temperature in Mill 2 was low due to a heating problem (16.7 ± 0.04°C, range: 6.2-25.6°C) and this would have reduced beetle movement. The temperatures in Mills 1 and 3 were higher however (Mill 1: 24.6 ± 0.03°C, range: 19.4-30.7°C; Mill 3: 28.1 ± 0.05°C, range: 16.8-37.0°C).

The number of beetles caught in the traps the day before and during sampling was compared to the number of beetles inside the corresponding rollstand. In no case was this correlation significant ($p>0.05$; Figure 4.5). Since no beetles were caught in Mill 1, no correlation was possible in that case. When the number of tracks in the front of each rollstand was compared in a similar way, the correlation was significant for Mill 2 only ($r^2=0.28$, $p=0.03$; Figure 4.6). When correlations were run comparing the number of beetles inside sample areas versus the numbers in rollstands (sample area versus internal total of rollstand - sample area), there was no consistent correlation across mills (Table 4.5).

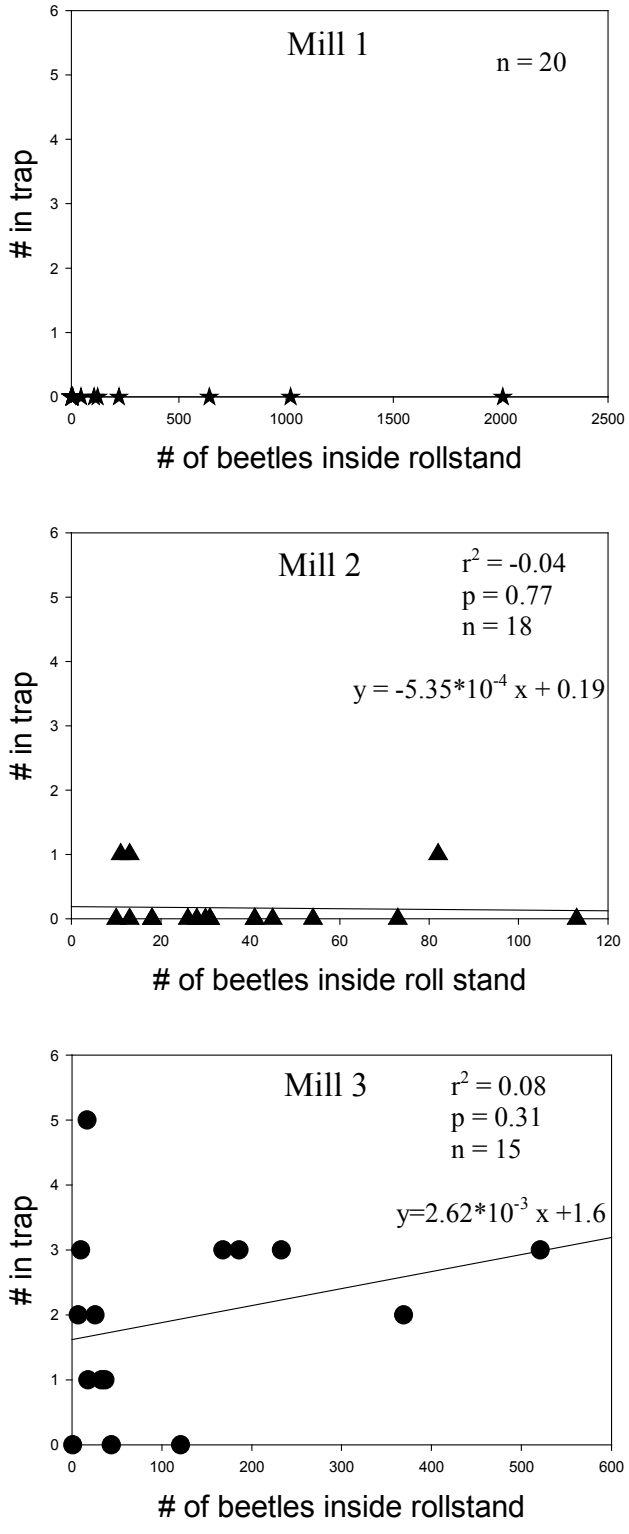

Figure 4.5. Correlations between the number of beetles caught in monitoring traps placed outside rollstands in flour mills, and the number of beetles inside the rollstands.

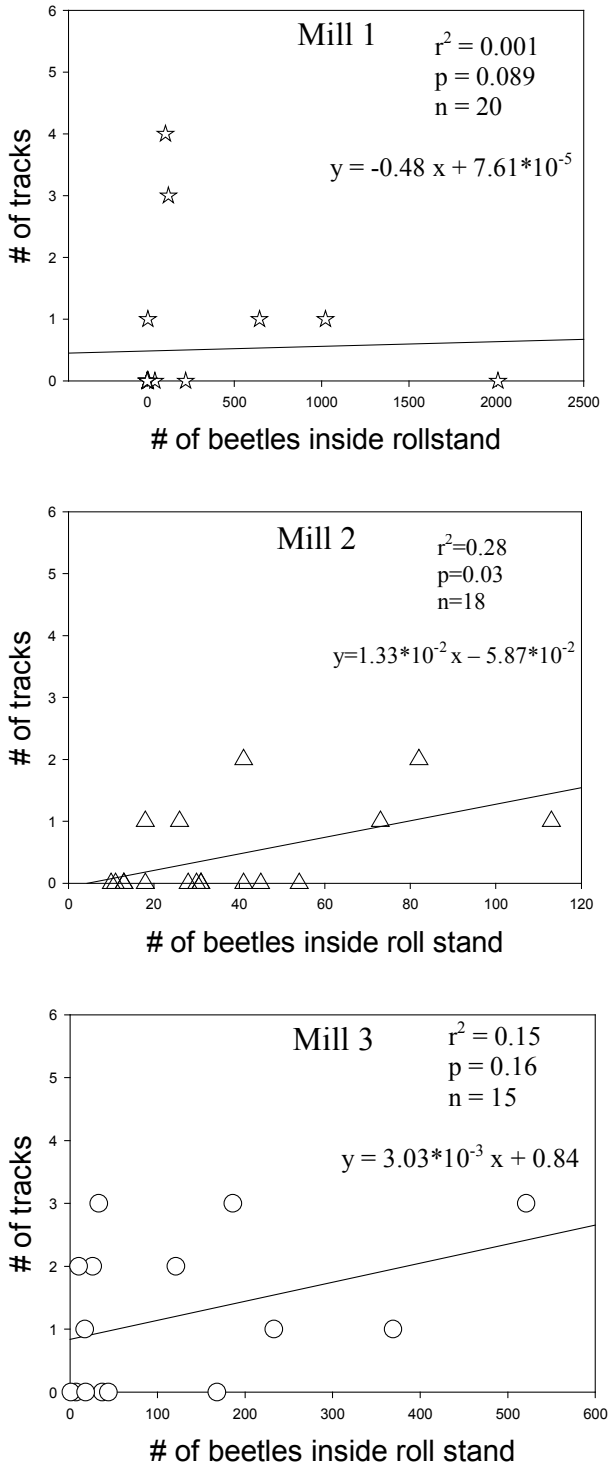

Figure 4.6. Correlations between the number of beetle tracks in front of rollstands in flour mills, and the number of beetles inside the rollstands.

Table 4.5. Correlations between the number of beetles in each sample area of a rollstand, compared to the total number of beetles in that rollstand minus the area. Significant results are bolded. The number in brackets is the number in the sample.

	Mill 1 (20)		Mill 2 (18)		Mill 3 (15)	
Area of rollstand	r^2	p	r^2	p	r^2	p
Bottom door (B)	**0.58**	**0.01**	0.31	0.21	0.45	0.09
Dead spots on outlet hopper (C)	0.05	0.83	0.24	0.34	**0.57**	**0.03**
Guards, brushes, panels and bolts (D)	**0.96**	**<0.001**	0.15	0.56	0.39	0.15
Dead spot under the top door (E)	0.36	0.11	0.07	0.79	**0.65**	**0.01**
Area between feed rolls (F)	0.28	0.22	0.16	0.53	0.30	0.28
Dead spots in upper inside corner of rollstand (G)	0.36	0.12	0.41	0.09	0.09	0.76
Right-hand suction port on top of rollstand (H)	0.19	0.40	0.25	0.32	0.47	0.08

In Mill 1, beetles were identified to species (either *T. confusum* or *T. castaneum*; Table 4.6). Only 26% of identified beetle were *T. confusum.* However, the majority of identified beetles (72%) came from two rollstands in the 2nd middlings flour mill stage which contained exclusively *T. castaneum*. More rollstands harboured *T. confusum* than *T. castaneum*. When rollstands containing less than 45 beetles are excluded, 7 of the 20 rollstands sampled are left; 3 of these rollstands had only *T. castaneum*, 1 had only *T. confusum* and the remaining 3 rollstands had 94-100% *T. confusum* (Table 4.6).

Table 4.6. The percentage of *Tribolium* beetles in rollstands in Mill 1 that were *Tribolium confusum*. Twenty rollstands were sampled; rollstands containing no beetles are excluded from the table. The values seen are from individual rollstands; they are not means.

Milling stage	Substage	% of *Tribolium confusum* found in rollstand	Total # of *Tribolium* beetles in rollstand
Breaks	2nd	50	2
	3rd	100	6
		100	3
		100	4
		100	1
		50	2
	4th	100	3
Middlings	1st	94	640
		99	219
		99	120
		100	104
	2nd	0	1016
		0	1909
	3rd	50	6
		0	45

Discussion

There were significant correlations between pupae and adult counts for both flour sampling areas and individual rollstands. It is difficult to tell exactly how long populations have been established in the machines with the present data. Immature stage populations of *T. castaneum* oscillate widely and regularly (approximately every month) in laboratory cultures, while adult population numbers tend to reach an equilibrium within the first few weeks (Desharnais and Liu, 1987). However, the populations must have been established for over a month, since developmental time from egg to pupae is usually less than 30 days for both *T. castaneum* and *T. confusum* (Rees, 2004; Sokoloff, 1974).

Most of the beetles within the rollstands were in the feed-gate area (22-82% of total beetle populations depending on mill). This area is difficult to access, making it challenging and time-consuming to clean. In terms of easy-to-access areas, the bottom door was responsible for 2-17% of beetle infestation inside the machines and the "dead spot" on the outlet hopper accounted for 11-30%. When these areas are added together, their beetle populations account for 13-43% of the overall number of beetles inside the rollstands. If one were to also clean the brushes and the dead spot under the top door, 16-51% of the beetle populations in this study would be eliminated. Cleaning easy-to-access areas could therefore eliminate a large number of the beetles present within the rollstands.

Beetles may be more prevalent in middlings rollstands because of increased survival rates rather than increased reproductive output. Smallman and Loschiavo (1952) report that *T. confusum* develop fastest and have more progeny on tailings and low-grade flour than on middlings or break-stage flour. They found an inverse relationship between

the number of progeny produced and the survival rates of adults however; after six months more adults survived on middlings and break-stage flour. There may also be more beetles in middlings stage rollstands because the environmental conditions inside those particular machines are more favourable for *Tribolium* beetles; milling staff indicated that the flour in one of the most infested middlings rollstands in Mill 1 was often moist.

Trap monitoring and track counting were both ineffective methods of determining which machines were most infested. This could be because there are few beetles leaving and entering the machines. Movement into and within a rollstand is probably perilous for beetles, as the movement of flour through the machine is rapid and forceful, and there is a significant risk of being crushed by the many moving parts of the rollstand. A few beetles, safely finding harbourage and food in the rollstands by chance, could be responsible for the established populations seen in this study.

The numbers of beetles caught in trap was quite low in this study. Such results contrast greatly with the numbers caught in pheromone traps for other species of stored product insects. Pierce (1994) used pheromone traps to control Indian meal moth and cigarette beetle populations in a food warehouse, and reports a 96% decrease in moth populations and 99% decrease in cigarette beetle populations. The overall low numbers of insects caught in these pheromone traps may have to do with the pheromone itself (4,8-dimethyldecanal, racemic mixture); both *T. confusum* and *T. castaneum* are attracted to it in a laboratory setting (Suzuki and Mori, 1983) but it may not be attractive enough in a flour mill setting to make short-term trap monitoring useful (Mullins, personal communication). The traps combine food bait (cereal oil) and pheromone to increase

attractiveness, but even with this added attractant the traps do not catch many beetles (see Chapter 5). Further research is needed to increase the efficacy of these traps.

Few of the rollstands contained both species, and those that did contained a very small percentage of *T. confusum*. There may not have been much species overlap in the rollstands because *T. confusum* may not often inhabit rollstands. This species cannot fly (Sokoloff, 1972) and that would make it difficult for a *T. confusum* beetle to reach areas deep within the equipment. *Tribolium confusum* is also less likely to seek out and exploit new resources than *T. castaneum* (Ziegler, 1976), so *T. confusum* populations are more likely to be found in more accessible areas than rollstand interiors. It is also possible that there were small numbers *T. confusum* present in the rollstands at some point, but they died before sampling. Since *T. confusum* burrow less than *T. castaneum* and disperse more from the surface of fresh flour (Sokoloff, 1974; Ogden, 1970; Ghent, 1963), *T. confusum* that end up inside a rollstand are more likely to wander around and be killed by the flour stream or the grinding equipment present within a rollstand. If a few *T. confusum* manage to navigate their way through a rollstand and found a population, there may not be enough genetic variation in the population for it to survive (Mayr, 1963).

It is also possible that *T. castaneum* and *T. confusum* were present together, entered into competition with each other and *T. castaneum* won. Park (1948, 1954, 1957) report that these two species cannot co-exist, and that one species always eliminates the other when the two species are put together into vials. However, *T. confusum* and *T. castaneum* often co-existed for years in Park's studies (as opposed to a maximum of six months here due to twice-yearly fumigations in Mill 1), and his studies were done under

much higher densities than the densities seen here. Whether these two species can co-exist together in mills is examined more thoroughly in Chapter 5.

While measurements of beetle activity outside the rollstands were not found to be useful in this chapter, targeted cleaning of rollstands may be of significant use in mills. Cleaning easy-to-access areas could have a significant impact on the established insect populations seen here. Also, cleaning two 2nd middlings rollstands in Mill 1 would knock down 72% of the overall beetle populations reported. These results indicate the importance of further scientific study into beetle distributions in mills. Only by thoroughly understanding the biology and behaviour of insect pests will one be able to employ successful and economical IPM practices.

Chapter 5: Sampling *Tribolium confusum* and *Tribolium castaneum* in mill and laboratory settings; differences in trap efficacy and behaviour between strains and species

Abstract

This chapter discusses differences in mill and laboratory strains of *Tribolium confusum* and *T. castaneum*. Monitoring traps showed low efficacy (trap catch) in both mill and laboratory settings. In a simulated warehouse experiment, mill-strain beetles were caught less often in traps than laboratory strains, and *T. confusum* was caught less often than *T. castaneum*. *Tribolium confusum* and *T. castaneum* were found together in all samples taken from a Canadian flour mill. The ratio of the two species in the samples was compared to that found in traps, revealing that *T. confusum* was caught less often than *T. castaneum*. In laboratory studies, beetles directly from a mill moved slower than beetles from a laboratory culture and this response was shown to be phenotypical. Mill-strain and laboratory-strain beetles also differed in burrowing tendencies in the laboratory.

Introduction

There is an increased need for alternatives to chemical control in food processing facilities. Consumers have become more wary of the health and environmental effects of insecticides and demand alternatives. Non-chemical control methods are often too costly to be economically practical however. Integrated pest management (IPM) helps to address this issue. In IPM one controls for pest populations only when the economic damage of the pests exceeds the cost of control, an approach that makes non-chemical

alternatives to pest control more accessible and economical (Subramanyam and Hagstrum, 2000).

Pest population sampling is a critical part of IPM. Pheromone traps can be helpful monitoring tools. A popular pheromone trap in Canadian flour mills is the Dome® Trap (Fields, personal correspondence; Mullen et al., 1992). This trap is baited with an isomeric mixture of 4R,8R-(-)- and 4R,4S-(+)-4,8-dimethyldecanal, the aggregation pheromone of *Tribolium confusum* and *T. castaneum* (Suzuki et al., 1984) and food attractants in the form of cereal oil. The number of beetles caught in the traps is considered a relative measure of the population in the mill, with large increases in trap catch considered a sign that the population in the mill has increased (Trécé Inc. and Insect Monitoring Systems and Pheromones, 2007).

Pheromone trap monitoring has limitations however. Trap catches can be influenced by many factors: temperature, lighting, the type of lure used in the traps and the availability of food (Phillips, 1997; Toews et al., 2005; Burkholder, 1985). Species and strain differences that affect trap catches have rarely been studied. Also, the relationship between absolute population sizes and the numbers of beetles caught in traps is not understood (Phillips et al., 2000). Currently, many food processing plants fumigate once or twice a year on pre-booked dates, regardless of the sizes of pest populations (Fields and White, 2002). An understanding of what the absolute numbers in the traps represent is needed in order to successfully implement IPM practices and end continued reliance on regularly-scheduled fumigations.

The following experiments examined effectiveness of pheromone trap monitoring with mill and laboratory strains of *Tribolium confusum* and *T. castaneum*. Several of

these experiments took place at a Canadian mill, since that is the "real world" for which these traps are designed. An experiment termed "the bullseye" studied the attractiveness of a close-range pheromone trap to individual *T. confusum*. The species ratios of *T. confusum/T. castaneum* found in flour samples taken from a mill were also compared to that seen in traps, to see if both species were trapped at equal rates. Finally, unbaited and baited pheromone traps were placed in a mill to test the effectiveness of the pheromone/oil combination used in the traps. In terms of laboratory studies, a warehouse recapture experiment and arena experiments tested if the two species and mill/laboratory strains were caught in traps at the same rate.

An in-depth knowledge of how pests interact with each other and how they behave around the commodity is also required for IPM. To this end, some studies on movement rates and burrowing tendencies were carried out on mill and laboratory beetles of both species. The species ratios found in the mill flour samples collected (see above paragraph) are discussed in terms of competition studies and other papers reporting *T. confusum* and *T. castaneum* present in the same mill.

Methods

All monitoring traps used in the following experiments were Dome® traps (Trécé Inc., Adair, Oklahoma) and temperatures were recorded using HOBO® data loggers (Onset Co., Bourne MA). Unless otherwise noted, statistics were run using SigmaStat® (Systat Software Inc., San Jose, California). Unbleached white flour is hereafter referred to as plain flour and unbleached white flour + 5% brewer's yeast is referred to as flour.

Mill experiments

Bullseye experiment

A circle was drawn on the fifth floor of a Canadian flour mill (Mill 1). This circle was 100 cm in diameter and had release spots marked at 10, 30, and 60 cm from the center of the circle, at the four compass points (for a total of 12 release spots; Figure 5.1). A monitoring trap was placed in the middle of the circle; depending on the treatment, this trap was either baited (pheromone and oil) or unbaited (no pheromone and no oil).

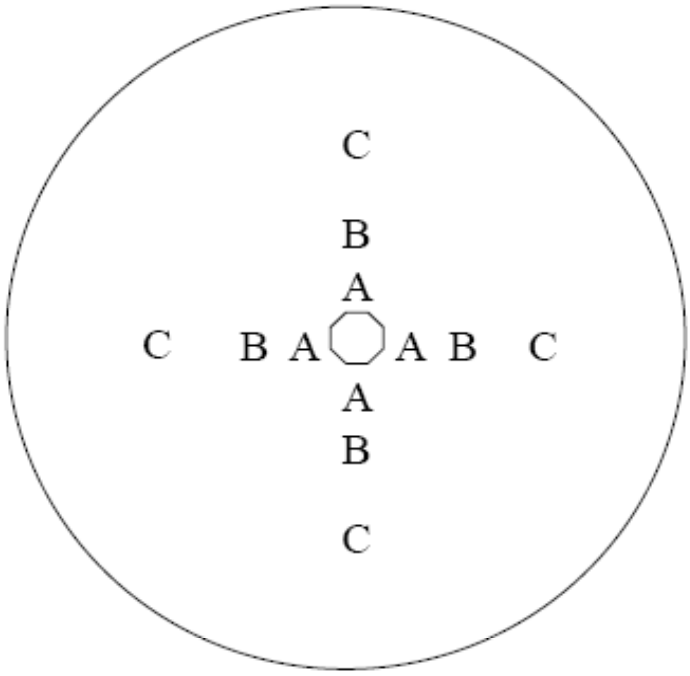

Figure 5.1. Schematic diagram of bullseye experiment. A monitoring trap was placed at the center of a circle. Beetles were released individually at point A, B or C and the number of beetles that touched or got caught in the trap was counted.

Virgin male *T. confusum* beetles from a laboratory strain were shipped to Mill 1 for this experiment. These males had been sexed as pupae (Hinton, 1942) and emerged shortly before being shipped. Beetles were placed in a large petri dish containing a sprinkle of plain flour and small pieces of filter paper for no more than 7 hours before being run in the experiment. The beetles were transferred one at a time to one of the 12 release spots (randomly chosen order) by picking up one beetle with the filter paper and placing the filter paper + beetle on the release spot; this was done to ensure a gentle release.

Once placed on the release spot, each beetle was timed until he had left the 100 cm outer limits of the circle, had been caught in the trap or 10 minutes had elapsed. A beetle was timed for 1 minute if he disappeared under the trap, and then the trap was checked to see if the beetle had been caught. All instances where the beetle touched the trap were noted; if a beetle was under the trap and was not caught after 1 minute, it was counted as a touch. One repetition of this experiment consisted of one beetle being released at every release spot.

There were 7 repetitions using a baited trap with new pheromone and oil. The trap was assembled the first day of the experiment and was used for the next three days while the repetitions were taking place. An additional 4 repetitions of the experiment were performed using a trap containing older pheromone and new oil; this trap was assembled 5 days prior to its use in this experiment and had previously caught beetles on the fourth floor of the mill. There were also 6 control repetitions using an unbaited trap. The ambient temperature and humidity were measured with a data logger attached to a wall in the center of the fifth floor; the mean temperature and humidity ± SEM was 31.2 ±

0.06°C (28.7-34.0°C), 39.1 ± 0.13% RH (35.0-45.2% RH). The number of touches and the number caught in the traps under the different conditions were compared.

Baited versus unbaited monitoring traps

There were 10 monitoring traps in the packing plant of Mill 1; these traps were used as part of the mill's IPM program and contained oil and pheromone (i.e. were baited). Five traps were located on the first floor of the packing plant and 5 traps were on the fifth floor. All the beetles in the traps were counted and discarded, then ten traps containing no oil or pheromone (i.e. unbaited traps) were placed 1 m away from each of the baited traps. All traps were left for one week and the number of beetles caught was counted.

Sample collection from mill

Flour samples were taken from various areas in Mill 1 and the adjoining packing plant. These samples were taken during the week of 16-23 November 2006, while the baited versus unbaited monitoring trap study was underway. All samples were sifted using a 850 µm sieve and the collected beetles were identified as either *T. confusum* or *T. castaneum*. All samples containing less than 15 beetles were excluded from analysis; under 15 beetles was believed to be too small a sample size to get an unbiased species ratios.

In order to compare species ratios in flour samples to those in traps, the beetles caught in 30 traps on 4 different floors of Mill 1 were also collected and analysed to species (*T. confusum* or *T. castaneum*). Beetles were collected from traps daily from 18-

23 November 2006. Beetles were also collected from 10 traps in the packing plant on 24 November 2006, upon completion of the baited versus unbaited monitoring trap study.

Laboratory experiments

Warehouse release-recapture experiment

Tribolium confusum and *T. castaneum* beetles, unsexed and unaged, were painted using slightly diluted fluorescent orange paint (DayGlo Color Corp., Cleveland, Ohio). (This was to test the resistance of the paint to oil found in monitoring traps.) The beetles used were gathered from Canadian flour mills 2-7 months prior to this experiment; the *T. confusum* beetles used came from Mill 1 and the *T. castaneum* beetles came from Mill 2. The experiment took place in a warehouse at the Cereal Research Center (Agriculture and Agri-Food Canada, Winnipeg, Manitoba; Figure 5.2). The warehouse (16.1m×5.2m) was cleaned, potential harbourage sites were removed and a cold treatment (temperature minimum = -24.3°C) took place prior to the experiment to kill any pre-existing populations of *Tribolium* in the warehouse.

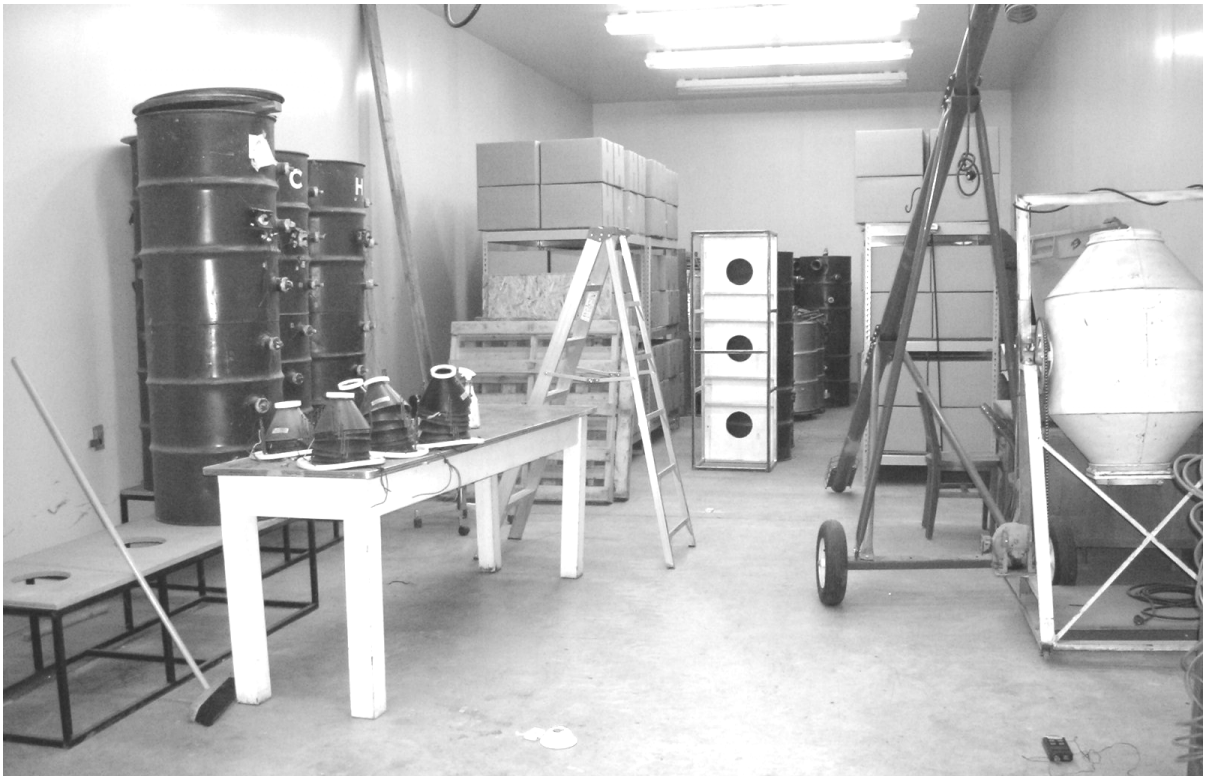

Figure 5.2. The warehouse at the Cereal Research Center (Agriculture and Agri-Food Canada, Winnipeg, Manitoba) used for a recapture experiment with *Tribolium confusum* and *Tribolium castaneum.*

Six monitoring traps were used in this experiment. Four of these traps were placed along the side walls (16.1m long), 2 traps per wall, and the remaining 2 traps were placed in the middle of the floor (Figure 5.3). Four days before the release of beetles, these traps were placed in the warehouse and checked for beetles. This ensured that the pheromone had a chance to age and also verified that the warehouse was free of *Tribolium* before the beetle release. The temperature and relative humidity throughout the warehouse and outside the warehouse during the experiment was measured. The lights in the warehouse were left on for the duration of the experiment, in order to simulate flour mill working conditions.

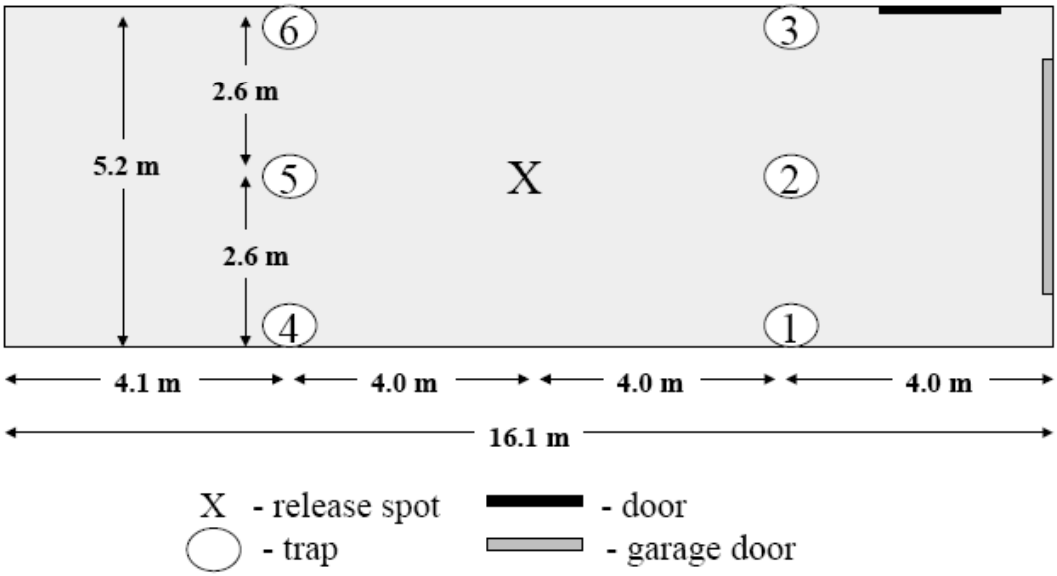

Figure 5.3. Schematic diagram of warehouse used for a recapture experiment, noting the placement of pheromone traps. *Tribolium confusum* and *Tribolium castaneum* were placed at the release spot and the numbers caught in the traps were recorded over a 10-day period. The numbers of the traps are given within the oval symbols. Diagram is approximately to scale.

Five hundred *T. confusum* and 500 *T. castaneum* beetles were brought to the warehouse in jars of plain flour. The beetles were sifted out of the flour and released gently; the sieve containing beetles was overturned on the release spot, in the middle of the warehouse, and left for 30 minutes at which point the beetles remaining on the sieve were brushed off. After the release, all six traps were checked for beetles daily for 10 days. All beetles caught were checked for paint and identified to species. Mortality during the experiment was negligible (less than 5%), as measured by bioassays (20 painted beetles in 12g of plain flour/bioassay, 3 bioassays/species).

The experiment described above showed that beetles did not remain marked with paint once caught in the traps. The experiment was repeated three more times without paint, resulting in a total of four trials of this experiment. Two of these trials (including the one described above) were for mill-strain beetles and two trials were for laboratory-strain beetles. Pheromone was aged for at least four days prior to each trial, except for lab-strain trial 1 in which new pheromone was added to the traps the day before the beetle release. For every trial, beetle populations were monitored after cold treatment using traps and the experiment did not commence if any beetles were caught. For average temperature and humidity data for all four trials, see Figures 5.4-5.7.

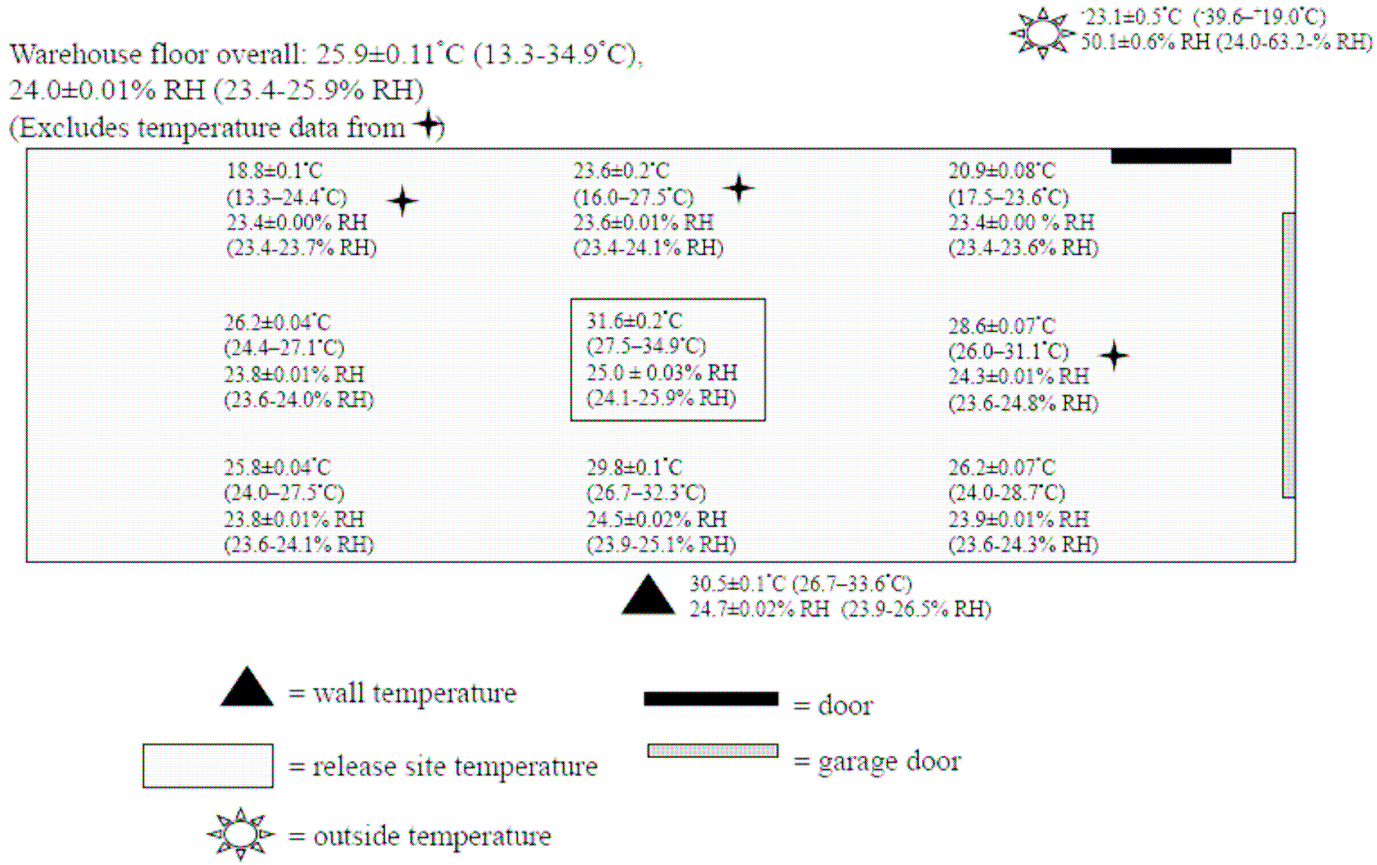

Figure 5.4. Average temperature and relative humidity (RH) measurements throughout a warehouse during the first trial of a recapture experiment using mill-strain *Tribolium confusum* and *Tribolium castaneum.* The trial took place 4-14 February 2007. Means ± SEM; ranges are in brackets. Diagram is approximately to scale.

✦ :temperatures were recorded 2-10 February only.

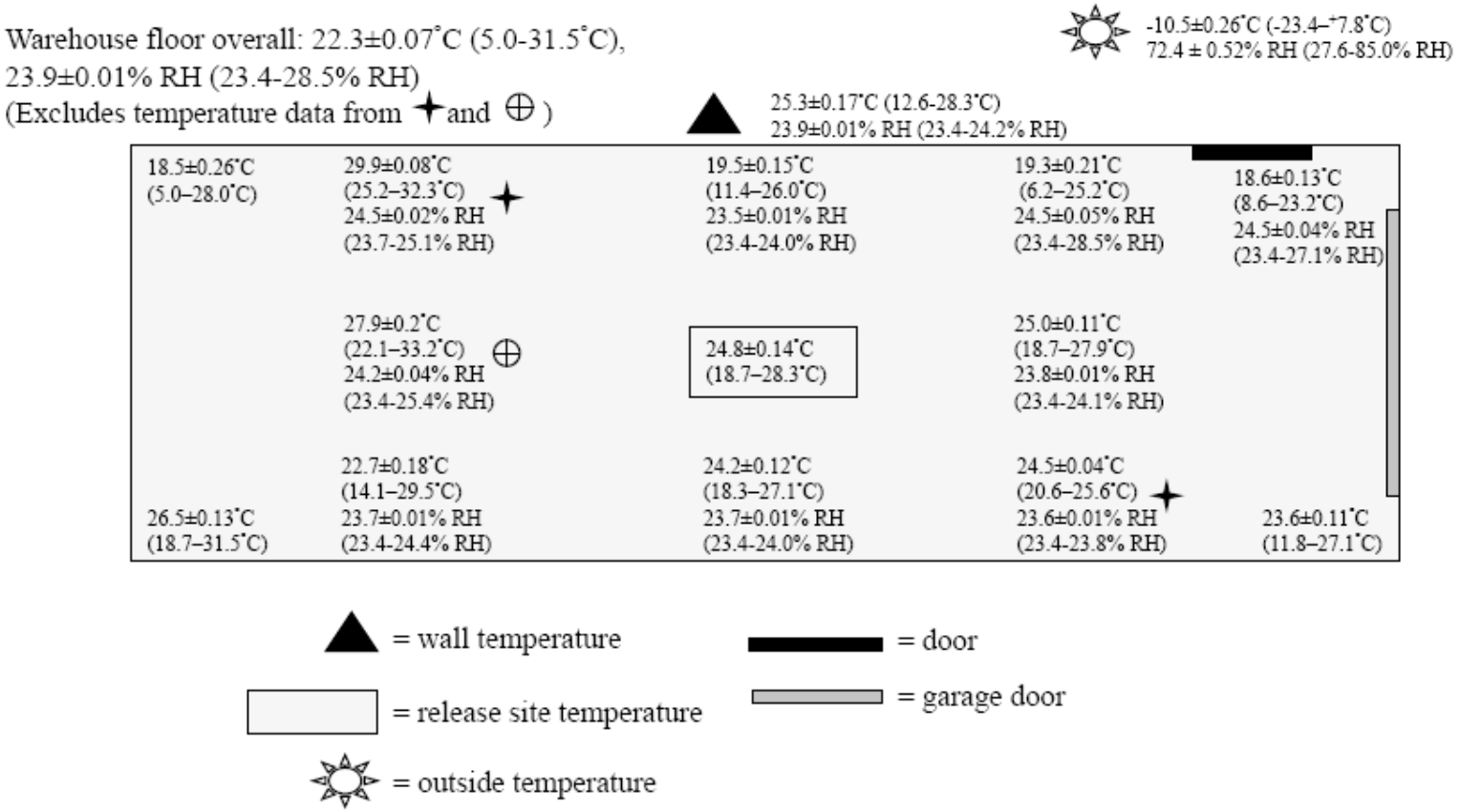

Figure 5.5. Average temperature and relative humidity (RH) measurements throughout a warehouse during the second trial of a recapture experiment using mill-strain *Tribolium confusum* and *Tribolium castaneum*. The trial took place 14-24 December 2007. Means ± SEM; ranges are in brackets. Diagram is approximately to scale.

✦ : temperatures were recorded 17-24 December only; ⊕ : temperatures were recorded 14-18 December only.

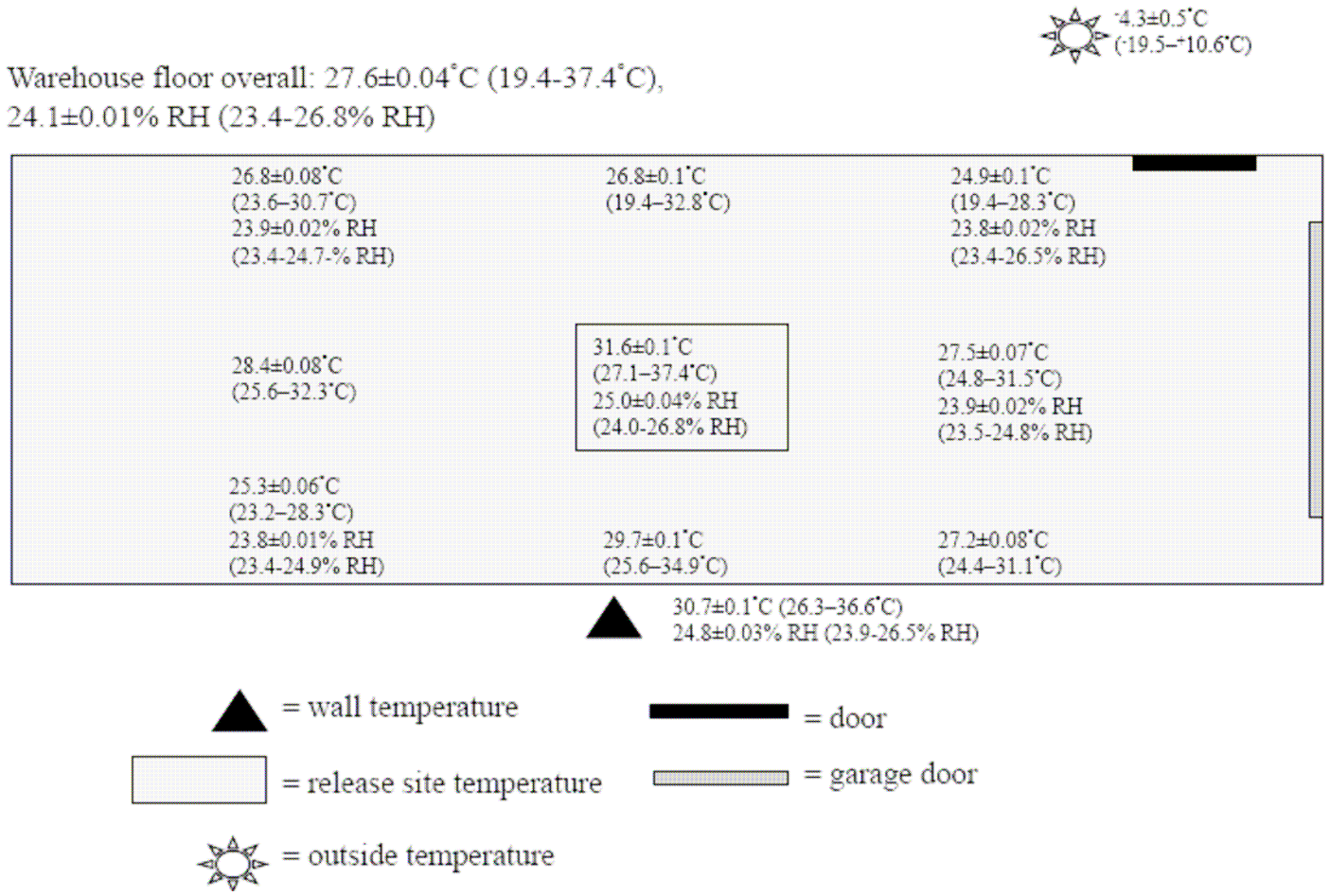

Figure 5.6. Average temperature and relative humidity (RH) measurements throughout a warehouse during the first trial of a recapture experiment using laboratory-strain *Tribolium confusum* and *Tribolium castaneum.* The trial took place 7-17 March 2007. Means ± SEM; ranges are in brackets. Diagram is approximately to scale.

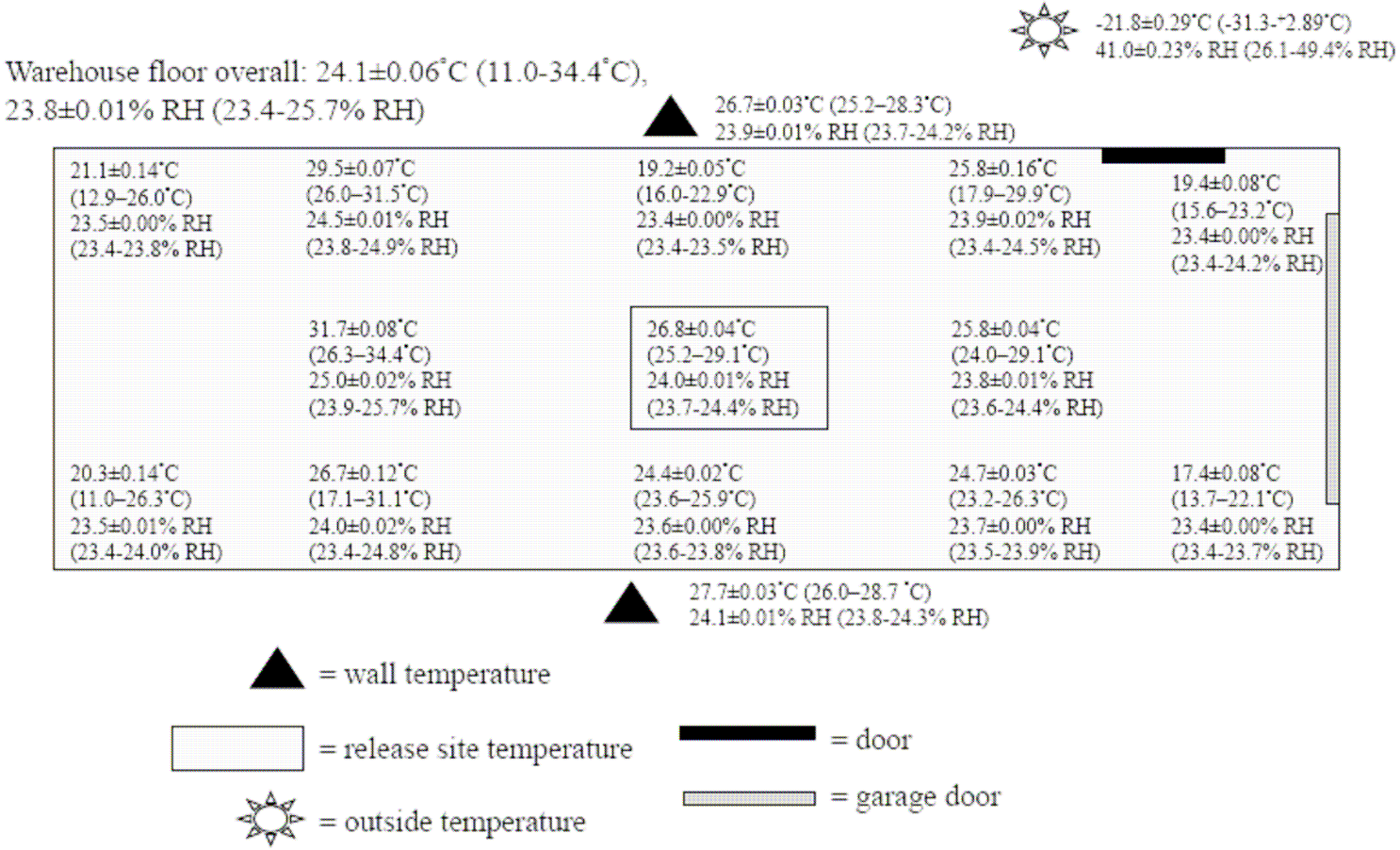

Figure 5.7. Average temperature and relative humidity (RH) measurements throughout a warehouse during the second trial of a recapture experiment using laboratory-strain *Tribolium confusum* and *Tribolium castaneum.* The trial took place 6-16 January 2008. Means ± SEM; ranges are in brackets. Diagram is approximately to scale.

The first mill-strain trial has been discussed in detail above. In the second mill-strain trial, *T. confusum* and *T. castaneum* beetles from Mill 1 were used. The minimum temperature during the cold treatment was -11.1°C. Mortality during the trial was negligible for *T. confusum*, but for *T. castaneum* 7 out of 20 beetles in one bioassay died, and 2 out of 20 died in another bioassay. The mortality seen with laboratory *T. castaneum* may have been caused by an infestation of the neogregarine *Farinocystis tribolii* Weiser which resisted a sanitation attempt; see egg sanitation procedure (page 112).

In the first lab-strain trial, the minimum temperature during the cold treatment was -6.3°C. Mortality was negligible during the trial (less than 5%), as measured by bioassays. In the second lab-strain trial, the minimum temperature during the cold treatment was -10.0°C. Mortality was negligible for *T. confusum* but *T. castaneum* mortality was 15% in two of the bioassays. (See above comments.)

For each trial, rearing conditions differed. For the first mill-strain trial, beetles were from two different mills (Mills 1 and 2), had been reared in the laboratory for different amounts of time (2-7 months) and were reared under different conditions. *Tribolium castaneum* had been successfully treated for mites (by rolling beetles in grain) two months prior to the experiment. In the second mill-strain trial, all beetles had been collected from Mill 1 less than 8 months beforehand and were reared the same way (200 g flour, 100 adults to start each culture, cultures created the same day). In the first lab-strain trial, beetles had been reared in the laboratory for years and were reared in the same fashion prior to the experiment (220 g flour, 60 adults to start each culture, cultures created the same day). In the second lab-strain trial, the beetles were once again reared the same way (200g flour, 100 adults to start each culture, cultures created the same day).

In this case however, the laboratory *T. confusum* had undergone a sanitation procedure 9 months prior to the experiment (see egg sanitation procedure).

Arena

This experiment was carried out in response to the results of the warehouse recapture experiment. It tested for differences in trap captures between strains and species of *T. confusum* and *T. castaneum,* using arenas containing no food or harbourage and one monitoring trap per arena. Beetles for this experiment were gathered from three different mills in Canada, or were taken from existing laboratory strains. There were a total of six different types of beetles used; *T. castaneum* (lab; Mill 1, 2 and 3) and *T. confusum* (lab; Mill 1). This experiment was repeated three times (trials 1-3). Trial 1 had four repetitions; trials 2 and 3 had three. The mill strains used were started no more than 6 months previous to trial 1 while the laboratory strains had been reared in the laboratory for many years prior to this experiment. The laboratory *T. confusum* used had undergone a sanitation procedure 6 months previous to trial 1 (see egg sanitation procedure).

There were 6 arenas used for this experiment, each consisting of a 1.2 m×1.2 m plywood base and metal sides. Fluon® (AGC Chemicals Americas, Inc., Bayonne NJ) was applied to the metal sides to keep beetles from escaping the arena. The arenas were placed in a grid pattern in a warehouse belonging to the Cereal Research Center (Winnipeg, Manitoba; Figure 5.8). The temperature and relative humidity inside the warehouse were measured by data loggers taped to the outside of each arena. The distance between each arena was 0.22 m - 0.35 m. The arenas were vacuumed in between repetitions.

Figure 5.8. The arena experiment. Two hundred beetles of various strains and species (mill or laboratory/*Tribolium confusum* or *Tribolium castaneum*) were placed in an arena with a monitoring trap for 24 hours, in the dark. The number of beetles in and beneath the trap was then recorded.

Two hundred beetles of each of the six types of beetles were released in the left-hand corner of each arena. A monitoring trap was placed in the center of each arena prior to the release of the insects; a new trap and pheromone lure was used for each repetition. For trials 1 and 2, traps all contained a pheromone septa and 3-4 drops of cereal oil (baited traps); for trial 3, unbaited traps (no oil or pheromone) were used. New traps were used for each repetition in order to avoid contamination. Once beetles were placed in the arenas, they were left for 24 hours in the dark. The number of beetles in the trap, under the trap and in the rest of the arena was then counted.

The beetles used in trials 2 and 3 were reared in the same manner for at least two months prior to the trial (200 g flour/culture, 100 adults to start each culture, all cultures started within a two-day period). The one exception to this rearing was laboratory *T. castaneum*; there was a culture of laboratory *T. castaneum* that had been reared like the other types of beetles but that culture remained diseased despite sanitation attempts. For trial 1, all beetles except *T. castaneum* were reared in this same manner; the culture of *T. castaneum* that had been reared for the experiment and was to be used contracted a disease, most likely *F. tribolii* (see egg sanitation procedure and warehouse methods). See Figure 5.9-5.11 for average temperatures and humidities during all three trials.

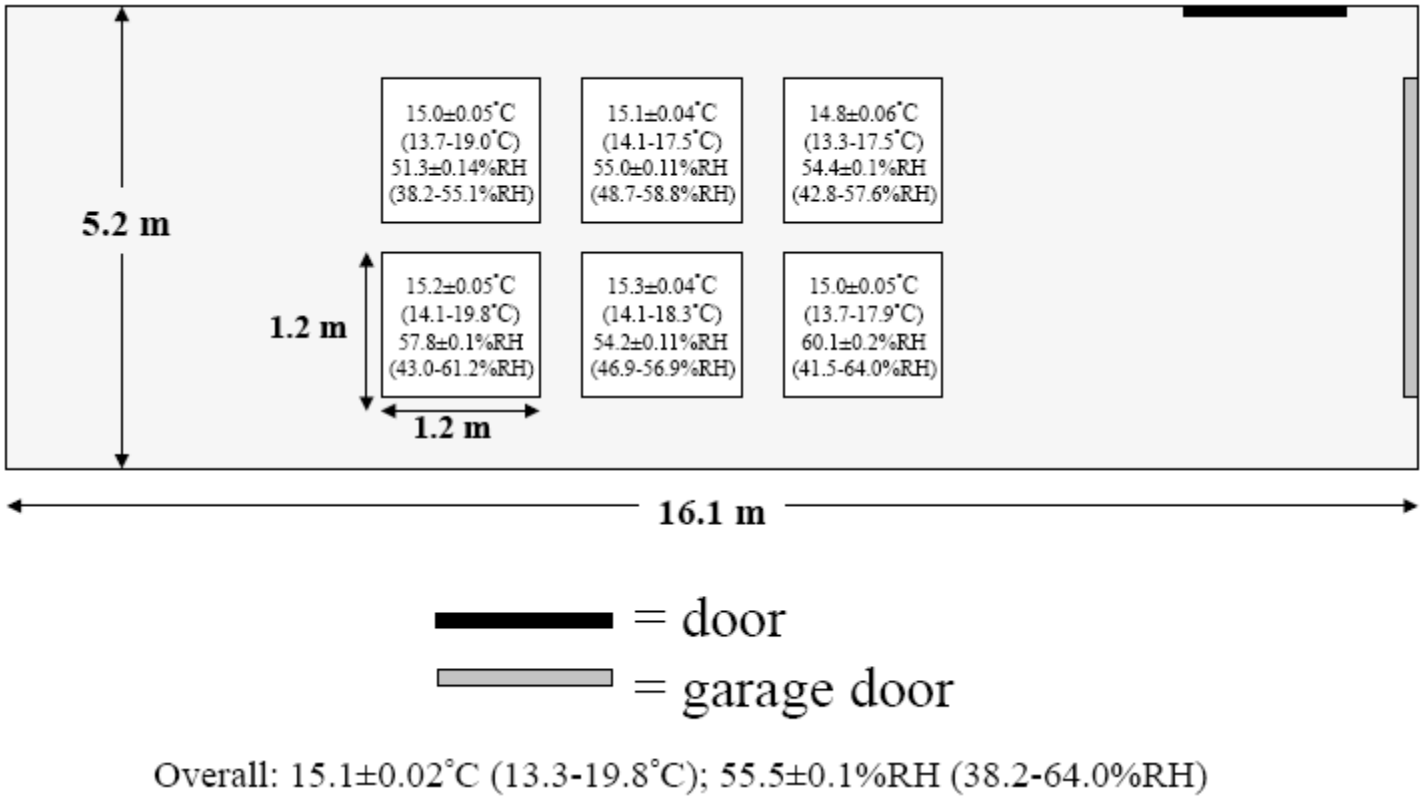

Figure 5.9. Temperatures during the first of three arena experiments. Two hundred *Tribolium* beetles of a specific strain and species were placed in each arena, a baited monitoring trap was placed in the middle of the arena and the number of beetles in each trap after 24 hours (dark conditions) was counted.

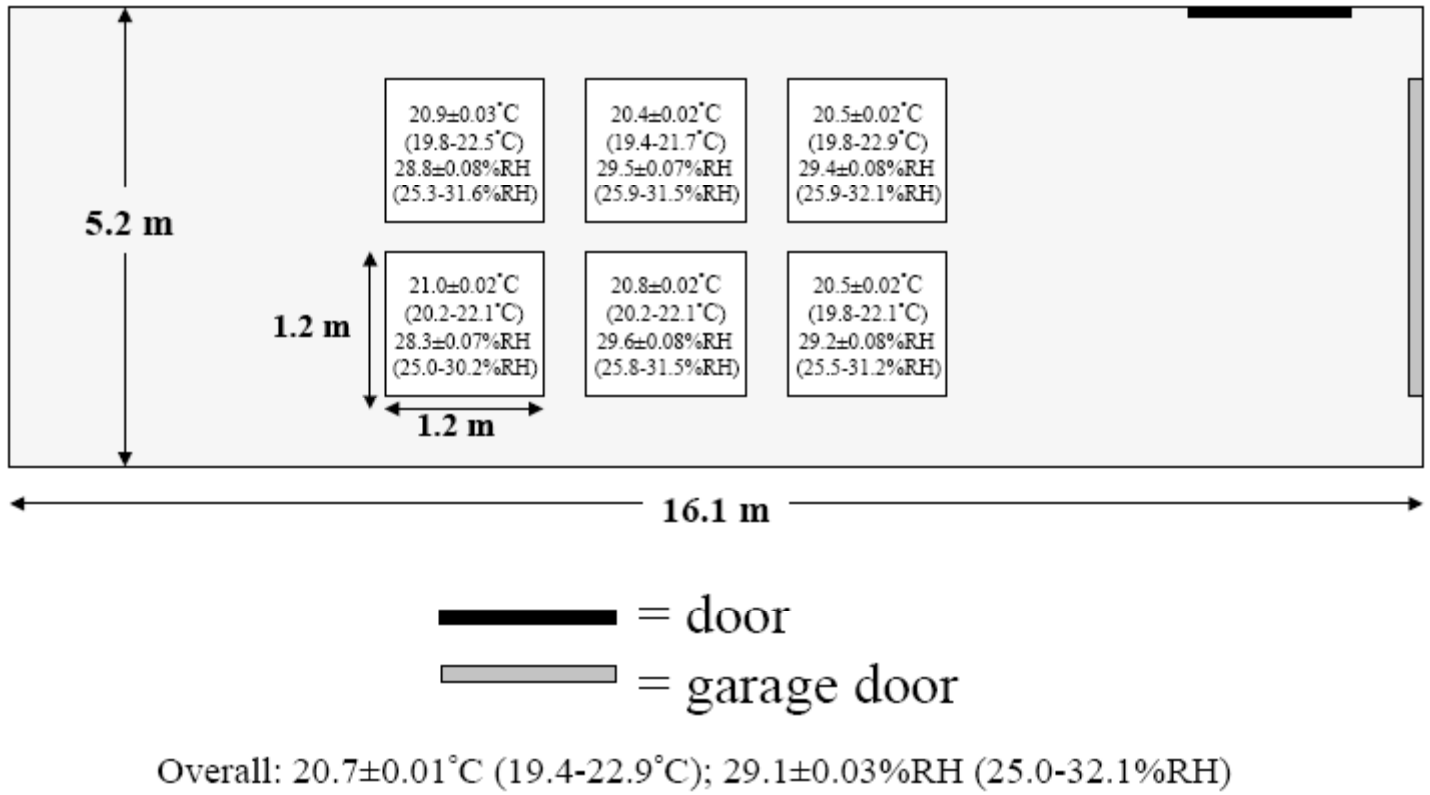

Figure 5.10. Temperatures during the second of three arena experiments. Two hundred *Tribolium* beetles of a specific strain and species were placed in each arena, a baited monitoring trap was placed in the middle of the arena and the number of beetles in each trap after 24 hours (dark conditions) was counted.

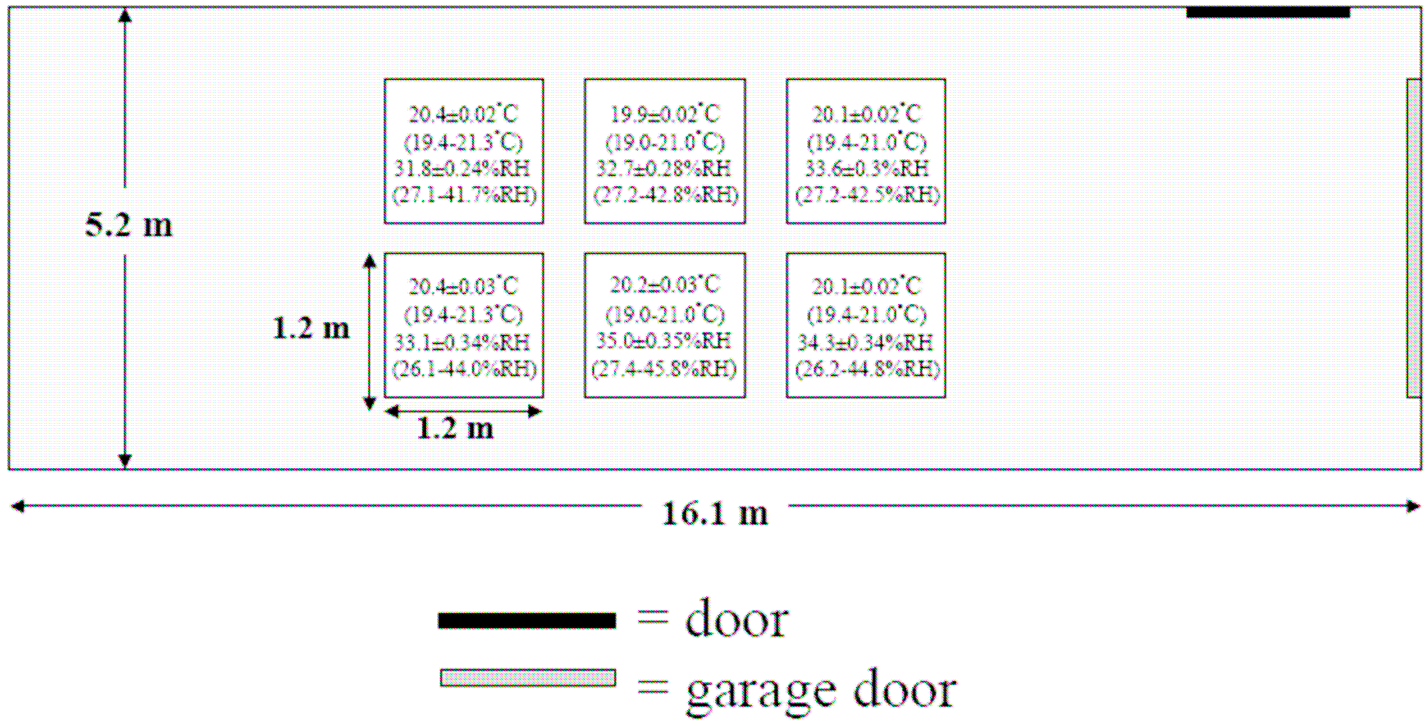

Figure 5.11. Temperatures during the third of three arena experiments. Two hundred *Tribolium* beetles of a specific strain and species were placed in each arena, an unbaited monitoring trap was placed in the middle of the arena and the number of beetles in each trap after 24 hours (dark conditions) was counted.

Movement rates - mill and laboratory beetles

Ten plastic rings (acrylonitrile-butadiene-styrene or ABS plastic), labelled 1-10, were placed around a Panasonic infrared camera (model V330APT; Vicon Industries Inc., Melville, New York), according to Rigaux et al. (2001). The sides of the rings were painted with Fluon® (AGC Chemicals Americas, Inc., Bayonne, New Jersey) to ensure that beetles did not escape from the rings. Black paper underneath the rings increased the visibility of the beetles during recording and facilitated beetle traction (Figure 5.12). The temperature and relative humidity (RH) during the two-day experiment were recorded using a data logger: the means ± SEM and ranges were 23.4 ± 0.01°C (22.9-25.2°C), 37.1 ± 0.13% RH (33.3-46.6% RH).

Figure 5.12. Setup to measure beetle movement rates. *Tribolium confusum* and *Tribolium castaneum* from either mill or laboratory strains were placed in the rings, one beetle per ring, and movement was recorded for one minute.

There were four different types of beetles used in this experiment: two species (*T. confusum* and *T. castaneum*) × two strains (mill and lab). Laboratory beetles were taken directly from laboratory cultures while mill beetles were taken directly from Mill 1. The mill beetles were placed in 1 L glass jars with approximately 250 g of flour between the time they were taken from the mill and separated by species, up until this experiment.

Beetles were inspected under low magnification for all antennal and leg parts then placed in vials, one beetle/vial. One capful of plain flour (approx. 0.3 g) was placed in each vial. The beetles within each of the vials were sifted out using a 600 μm sieve and gently brushed into the appropriate ring, by holding the sieve up at an angle and brushing the beetle upwards and into the ring. The replicate and order of placement for each vial was chosen randomly. There were a total of 60 beetles in this experiment with 10 beetles per replicate.

The lights in the infrared recording room were turned off, the door to the room was closed and the beetles were left undisturbed for 1 hour. Infrared recording of each beetle's movements commenced after that hour had passed. Using a remote control placed outside the camera room, the camera panned until ring 1 became visible. The movements of the beetle in this dish were recorded for one minute, then the camera panned to ring 2 etc. Once recording was finished, each beetle was immediately weighed and then frozen in order to determine sex (Hinton, 1942).

Rate of movement was measured by placing a sheet of acetate over a large TV screen, playing the recording and constantly marking the path of the beetle for a minute (Figure 5.13). The distance travelled in cm/min was determined by taping a string along the path of the beetle, measuring the string and then adjusting this measurement to reflect

the difference between the size of the ring shown on the TV and the actual size of the ring. A ruler was placed on the floor around the camera and videotaped to determine the scale.

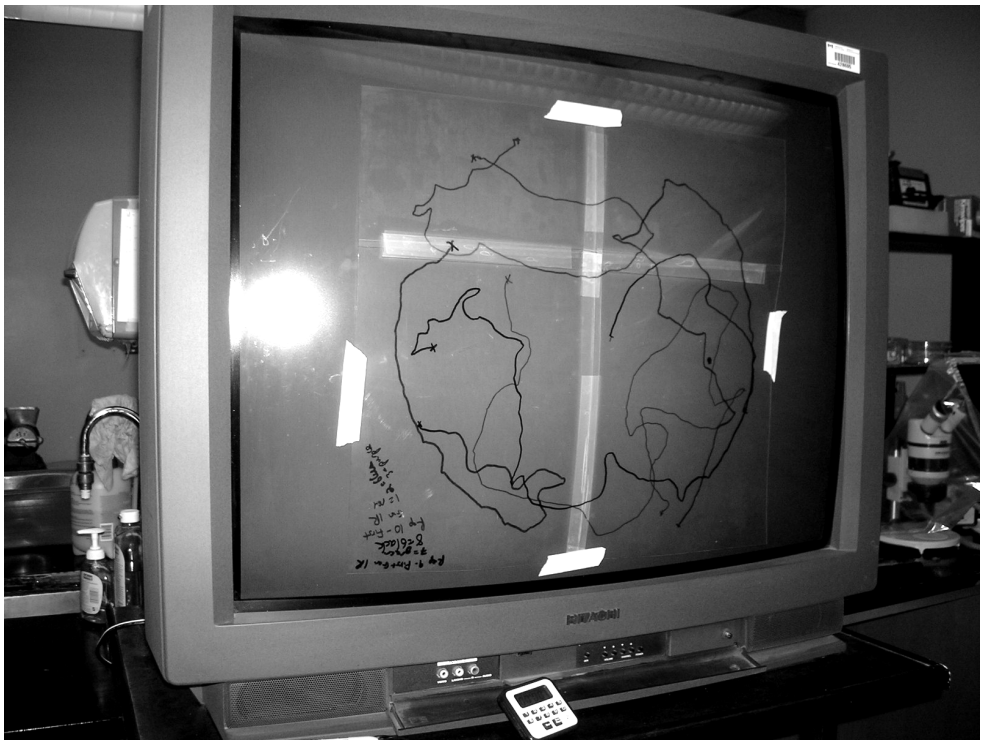

Figure 5.13. Setup used to measure rate of movement. A sheet of acetate was placed over a TV monitor and a timer (bottom middle) was used to time one minute.

Beetles were checked before and after the experiment to ensure they were the correct species (Bousquet, 1990). A plastic hose was connected to a 1000 µl pipette tip which had a hole in the side; this tip was in turn inserted into a 100 µl pipette tip. The hose was hooked up to a vacuum outlet. In order to "pick up" a beetle using this device, the vacuum was turned on, my thumb was placed over the hole in the 1000 µl tip and the entire device was placed directly over the back of a beetle. Once the beetle was attached to this device, the head and antenna of the beetle were examined under a microscope

without damaging the beetle. I took my thumb off the hole once inspection was complete and the beetle was released (Figure 5.14).

Figure 5.14. Device used to capture and hold live *Tribolium* beetles to species.

To determine of weight and rate of movement differences were due to the phenotype or genotype, this experiment was repeated using first-generation progeny of the beetles. Cultures were made of both mill and laboratory *T. castaneum* and *T. confusum*; 12 beetles of each type were placed in separate 32-ounce quart sealer jars, given the same amount of flour (approximately 250 g) and left in an incubator (30°C, 65% RH) until pupae were present (approximately 3 1/2 - 4 weeks). Pupae were sexed and reared to adulthood.

Since the beetles run in the original experiment were probably mated, the first-progeny beetles were mated prior to this experiment. Sixty beetles of each strain and sex

were spray-painted with orange fluorescent paint (DayGlo Color Corp., Cleveland, Ohio). They were left for a minimum of two hours to let the paint dry, after which they were individually checked for paint and 45 beetles were selected. These beetles were combined with 45 non-painted beetles of the opposing sex (same strain and species), given 230 g flour and left for three days. After this period, the spray-painted beetles were removed and the remaining beetles were placed in fresh medium, 2 g flour/beetle. The experiment took place the next day.

The protocol for this experiment was the same as the previous movement experiment, with a few modifications. Instead of 10 rings around the camera, there were 8. Also, there were a total of 120 beetles run in the experiment; 8 types of beetle (2 × sex/strain/species) and 15 repetitions. The average temperature and humidity during the experiment ± SEM was 17.5 ± 0.02°C (16.8-23.6°C); 37.4 ± 0.08% RH (32.5-46.9% RH).

Some beetles in both the first and second experiment were excluded from analysis because of technical problems during recording or because they escaped. When beetles were excluded from movement rate analysis, they were also excluded from weight analysis.

Burrowing - mill and laboratory beetles

This experiment tested for differences in burrowing behaviour between strains and species of *T. confusum* and *T. castaneum*. Beetles for this experiment were either gathered from one of three different mills in Canada (Mills 1-3) less than 7 months prior to this experiment, or were taken from existing laboratory strains. There were a total of

six different types of beetles used; *T. castaneum* from Mills 1-3, *T. confusum* from Mill 1, and laboratory *T. castaneum* and *T. confusum*.

All mill strain beetles and laboratory *T. confusum* were reared the same way for almost three months prior to the experiment (all 200 g flour/culture, 100 adults to start each culture, all cultures created within a two-day period). The laboratory *T. castaneum* used were not reared in the same fashion as the other beetles; there was a culture of laboratory *T. castaneum* that had been reared like the other types of beetles but that culture remained diseased despite sanitation attempts. The mill strains used were started no more than 6 months previous to this experiment. The laboratory *T. confusum* underwent a sanitation procedure 6 months previous to the experiment (see egg sanitation procedure).

Six tubes were used to measure burrowing distributions. Tubes were 100 cm tall with a diameter of 10 cm and were made from polyvinyl chloride pipe (PVC). There were horizontal slots every 10 cm of height and metal separators were fashioned to fit into these slots. Tape was used to cover the slots during the experiment. Holes in the tubes were covered using rubber stoppers (Figure 5.15).

Figure 5.15. Tubes used to measure beetle distribution throughout a 36cm height of flour. 200 *Tribolium* beetles of a known species and strain were placed on the top of the flour height and left in the dark for 24 hours.

Plain flour (1800 g) was placed into each of six tubes, resulting in a flour height of approximately 36cm. The tubes were secured vertically in an incubator (Figure 5.16) and left for at least 10 hours to allow the top, middle and bottom of the flour in these tubes to reach an equal temperature (29.1-29.5°C). Data loggers and external thermistors were used to verify that temperature had equalized.

Figure 5.16. Tubes secured inside an incubator. Tubes were left undisturbed and in the dark for 24 hours.

Two hundred beetles of each of the six types of beetles were randomly placed inside each of these tubes, on the surface of the flour (1 species/strain per tube). The tops were placed on the tubes and the beetles were left to burrow for 24 hours in the dark. At this end of this period, the tubes were removed from the incubator and metal separators were inserted into the pre-existing slots at heights of 10, 20 and 30 cm. This resulted in four separated sections of flour in each tube; the three bottom sections contained 10 cm of flour while the top section contained 6 cm, for the total height of 36 cm of flour.

Flour from each section of tube was weighed, the flour was sifted using a 850 µm sieve and the beetles were counted. Metal separators were removed one by one as the sections were weighed and sifted for beetles. A generalized linear model procedure was run with SAS® (SAS Institute Inc., Cary, North Carolina) to compare beetle distributions within the flour. To ensure that the amount of flour in each section was approximately equal, the weight of the flour in the three bottom (10 cm) sections was averaged for each tube and each repetition, and each section was compared to this average. In no case was any section more than ± 7.1% from the average.

Egg sanitation procedure

Eggs were sanitized to reduce *F. tribolii* infestations in beetle cultures. This was done by pre-sifting flour using a 150 µm sieve and placing 200 insects from each diseased strain in 100 g of this pre-sifted flour. After three days, a 180 µm sieve was used to retrieve the eggs.

Filter paper (#1, 110 mm) was wetted with distilled water and placed inside a vacuum flask (Figure 5.17). Eggs were soaked for 10 minutes in 3% aqueous solution of gluteraldehyde and then poured onto the filter paper. The eggs were rinsed three times with distilled water and then left for 15 minutes with the vacuum suction on to facilitate drying. The filter paper was then placed inside a petri dish, a sprinkle of plain flour was added and the petri dish was placed in an incubator (30°C, 65% RH). After three days, the filter paper was placed in 200g of flour. Once beetles emerged, new cultures were made.

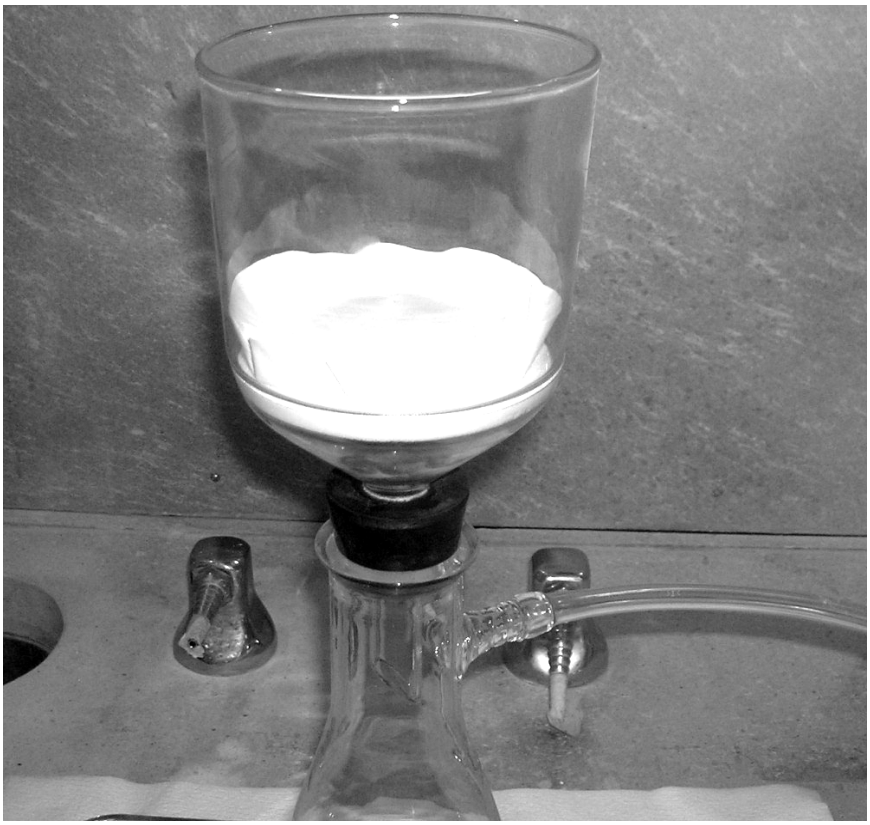

Figure 5.17. Setup used to sanitize *Tribolium* spp. eggs.

Results

Mill experiments

Bullseye experiment

When a trap with new pheromone and oil was placed in the middle of the circle, only 2 beetles out of 84 were caught in the trap, and these two beetles were released from either 10 cm or 60 cm away from the trap. When a trap with 5-day-old pheromone and new oil was used, no beetles were caught; this was the same as the unbaited trap. (Table 5.1.) A chi-square test was run comparing the overall number of beetles that touched or did not touch the trap in each treatment (new pheromone, older pheromone, unbaited trap). There was no significant difference between the treatments (chi-square = 1.467, p=0.48).

Table 5.1. The number of beetles that were caught in or touched a trap in a flour mill. All beetles were released individually less than 60 cm away from the trap.

Treatment	Age of pheromone, oil	Release distance from center of trap	# caught in trap	# of touches	# of beetles not responding
Pheromone, oil	New pheromone (84)	10 cm	1	4	79
		30 cm	0	3	81
		60 cm	1	0	83
	5-day-old pheromone (48)	10 cm	0	4	44
		30 cm	0	0	48
		60 cm	0	0	48
No pheromone, no oil	N/A (72)	10 cm	0	8	64
		30 cm	0	1	71
		60 cm	0	1	71

Number in brackets indicates number of beetles in the treatment.

Baited versus unbaited monitoring traps

A total of 20 traps were used; 10 were baited, 10 were unbaited. There were no beetles caught in any unbaited trap while baited traps did catch beetles (Table 5.2). A paired t-test [log (x+1) transformation] showed that baited traps caught significantly more beetles than unbaited ($t = 3.1$, $p = 0.01$).

Table 5.2. The number of beetles caught in traps containing oil/pheromone and no oil/no pheromone. The experiment took place at a Canadian flour mill.

Location of trap	# of beetles caught	
	Trap with pheromone, oil ¶	Corresponding control trap (no pheromone, no oil) ¶
1st floor packing plant	12	0
	0	0
	2	0
	0	0
	0	0
5th floor packing plant	0	0
	1	0
	3	0
	3	0
	2	0
Total	23	0

¶ Treatments are significantly different ($p<0.05$)

Sample collection from mill

The packing plant samples had a significantly higher percentage of *T. confusum* overall than the mill samples (Table 5.3; chi-square=71.1, $p<0.01$ using Bonferroni inequality, Yate's correction used). There was also a difference in the percentage of *T. confusum* when samples from different sections of the mill were compared (Table 5.3; rollstand versus boots: chi-square=403.0, $p<0.01$ using Bonferroni inequality, Yate's correction used).

In contrast to the 91% *T. confusum* seen overall in the mill samples, only 45% of beetles caught in mill traps during the corresponding time were *T. confusum* (Table 5.4). Only 9% of the beetles caught in the packing plant traps were *T. confusum,* compared to 95% in the samples. The difference between the percentage of *T. confusum* found in samples and traps was significant when mill, packing plant and overall values were compared. (Mill samples versus traps: $z=3.4$, $p<0.01$ using Bonferroni inequality, Yate's correction used; packing plant samples versus traps: $z=14.2$, $p<0.01$ using Bonferroni inequality, Yate's correction used; overall samples versus traps: $z= 9.449$, $p<0.01$ using Bonferroni inequality, Yate's correction used; Figure 5.18).

Table 5.3. The percentage of beetles that were *Tribolium confusum* in flour samples taken from a flour mill. Samples are broken down according to milling stage. Beetles were collected 16-23 November 2006.

Description	Mill/packing plant	Milling stage	Substage	% of *Tribolium confusum*	Total # of beetles
Rollstands	Mill	Breaks	4th	84	89
		Middlings	1st	78	55
			4th	90	39
			8th	94	155
		Low Grades	2nd	95	656
		Unknown	Unknown	87	31
		Unknown	Unknown	97	195
		Total (rollstands)		93	1220
Boots	Mill	Breaks	1st	7	131
				88	24
			2nd	88	16
			3rd	60	15
			4th	27	33
		Sizings	fine	29	24
		Middlings	2nd	51	148
				78	93
		Low Grades	Bran duster	70	23
			Bran sifter	69	16
			quality	93	28
		Tailings	2nd	76	134
		Total for boots		55	685
Sieves	Mill	Unknown	Unknown	42	19
Sifters	Packing plant	Unknown	Unknown	88	33
				95	150
				95	372
		Total for sifters		95	555

Table 5.4. The percentage of beetles that were *Tribolium confusum* in monitoring traps in a Canadian flour mill and packing plant. Beetles were collected 18-24 November 2006.

Mill/packing plant	Location	% of *Tribolium confusum*	Total # of beetles
Mill	1st floor	50	10
	3rd floor	40	5
	4th floor	50	2
	5th floor and adjacent areas	33	3
	Total for mill	45	20
Packing plant	1st floor	14	14
	5th floor	0	9
	Total for packing plant	9	23

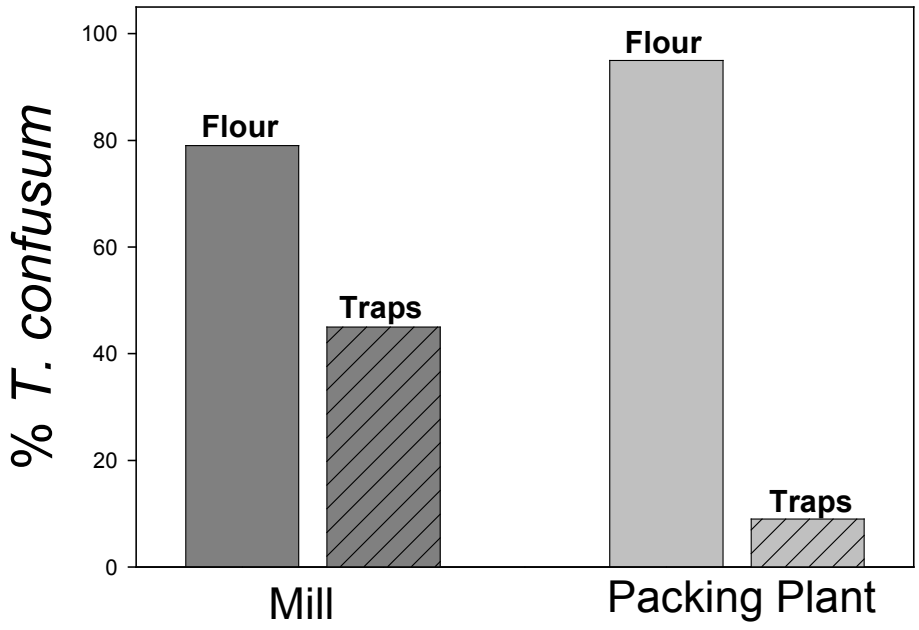

Figure 5.18. The percentage of beetles that were *Tribolium confusum* in pheromone traps and samples, taken from a flour mill and adjoining packing plant. There was a higher percentage of *Tribolium confusum* found in flour samples than in traps for mill, packing plant and overall ($p<0.01$ in each case).

Laboratory experiments

Warehouse recapture experiment

In terms of the mill-beetle trials, no beetles of either species were caught for the first day of trial 1 and for the first three days of trial 2. Also, no beetles were caught in Trap 2 during trial 2, although the temperature and humidity in this area were high enough for beetles to move (mean ± SEM: 25.0 ± 0.11°C, 18.7-27.9 °C; 23.6 ± 0.01% RH, 23.4-24.1% RH) and the temperatures in this area were mid-range compared to all other areas of the warehouse monitored with data loggers (Figure 5.5; see methods). No *Tribolium confusum* were caught during the entirety of trial 1 (Figure 5.19). Both species were caught in trial 2 but *T. confusum* was only caught on days 7, 8 and 10 of sampling (Figure 5.20). More *T. castaneum* than *T. confusum* were caught overall during both trials (trial 1: $z=6.4$, $p<0.01$ using Bonferroni's inequality, Yate's correction used; trial 2: $z=4.2$, $p<0.01$ using Bonferroni's inequality, Yate's correction used).

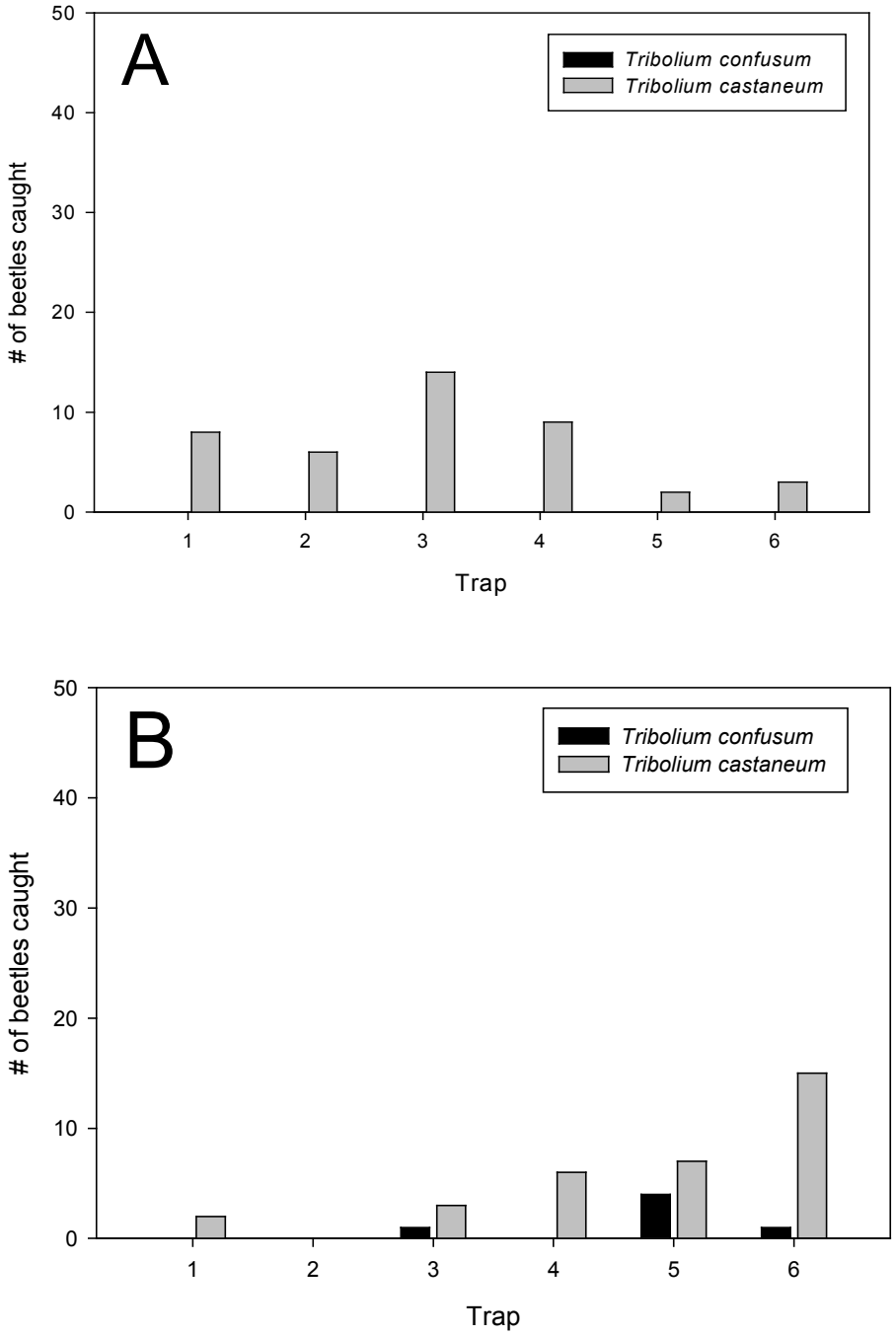

Figure 5.19. The number of beetles caught in 6 pheromone traps when 500 *Tribolium confusum* and 500 *Tribolium castaneum* beetles from mill strains were released into a warehouse (16.1m × 5.2m). A: trial 1, 4-14 February 2007; B: 14-24 December 2007.

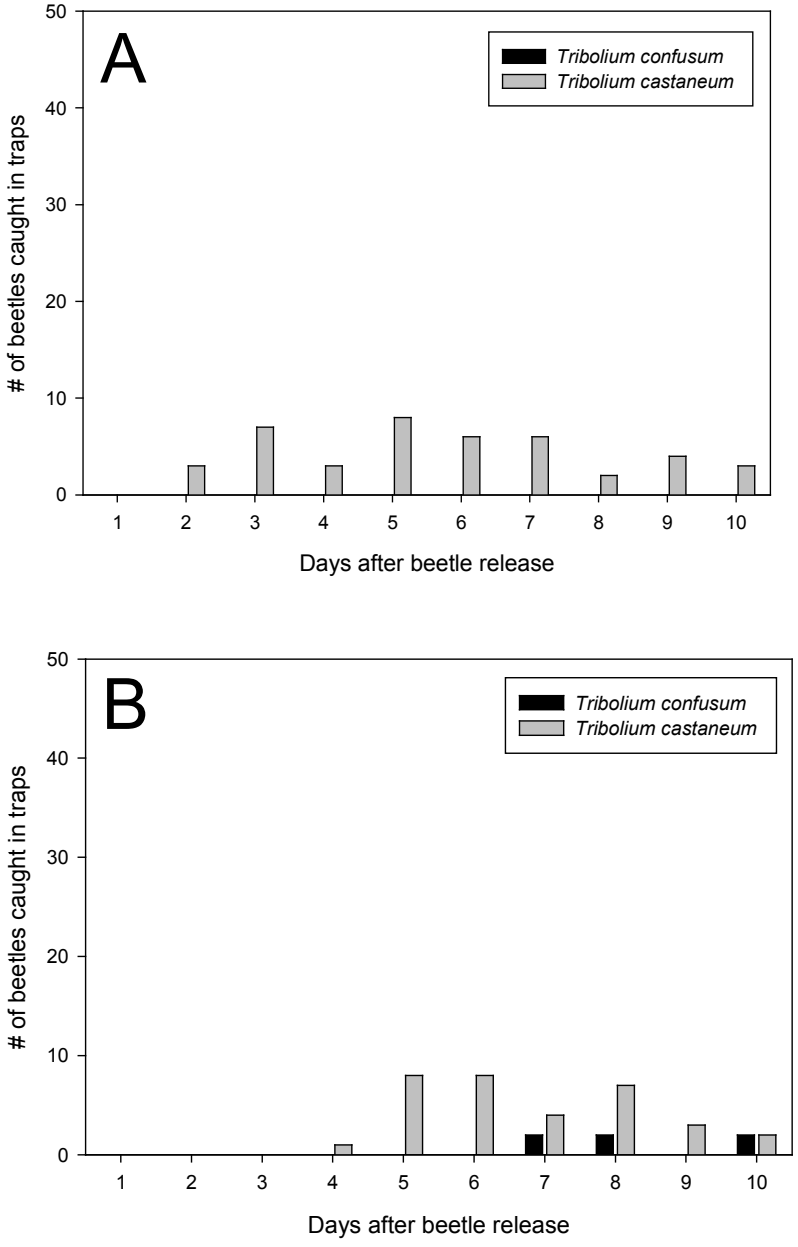

Figure 5.20. The number of beetles caught in pheromone traps over a 10-day period when 500 *Tribolium confusum* and 500 *Tribolium castaneum* beetles from mill strains were released into a warehouse (16.1m×5.2m). A: trial 1, 4-14 February 2007; B: 14-24 December 2007.

When laboratory beetles were released into the warehouse, both species were caught in every trap in both trials (Figure 5.21). In trial 1 lab-beetle release, no *T. confusum* were caught the first day of sampling. In trial 2, no *T. confusum* were caught on days 1 and 3 of sampling. (Figure 5.22). More *T. castaneum* than *T. confusum* were caught overall during both trials (trial 1: $z=2.9$, $p<0.01$ using Bonferroni's inequality, Yate's correction used; trial 2: $z=5.1$, $p<0.01$ using Bonferroni's inequality, Yate's correction used).

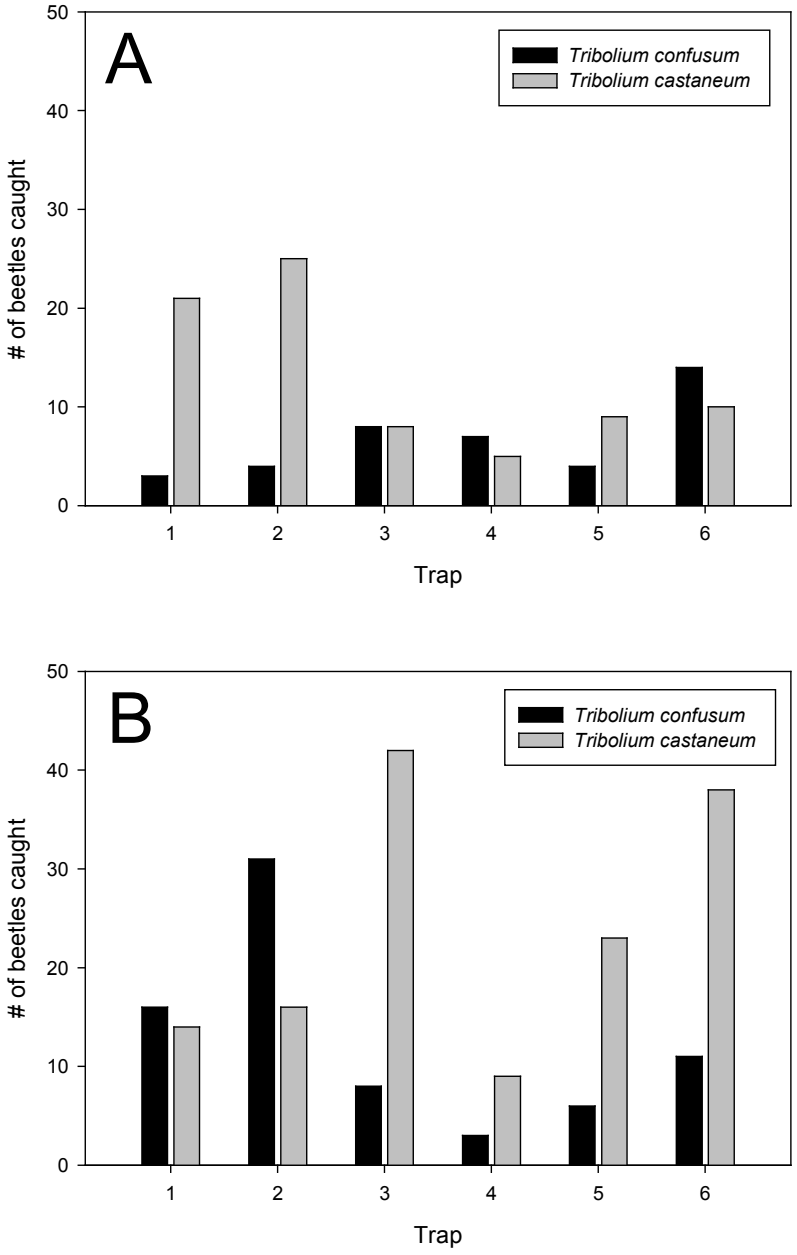

Figure 5.21. The number of beetles caught in 6 pheromone traps over a 10-day period when 500 *Tribolium confusum* and 500 *Tribolium castaneum* beetles from laboratory cultures were released into a warehouse (16.1m×5.2m). A: trial 1, 7-17 March 2007; B: 6-16 January 2008.

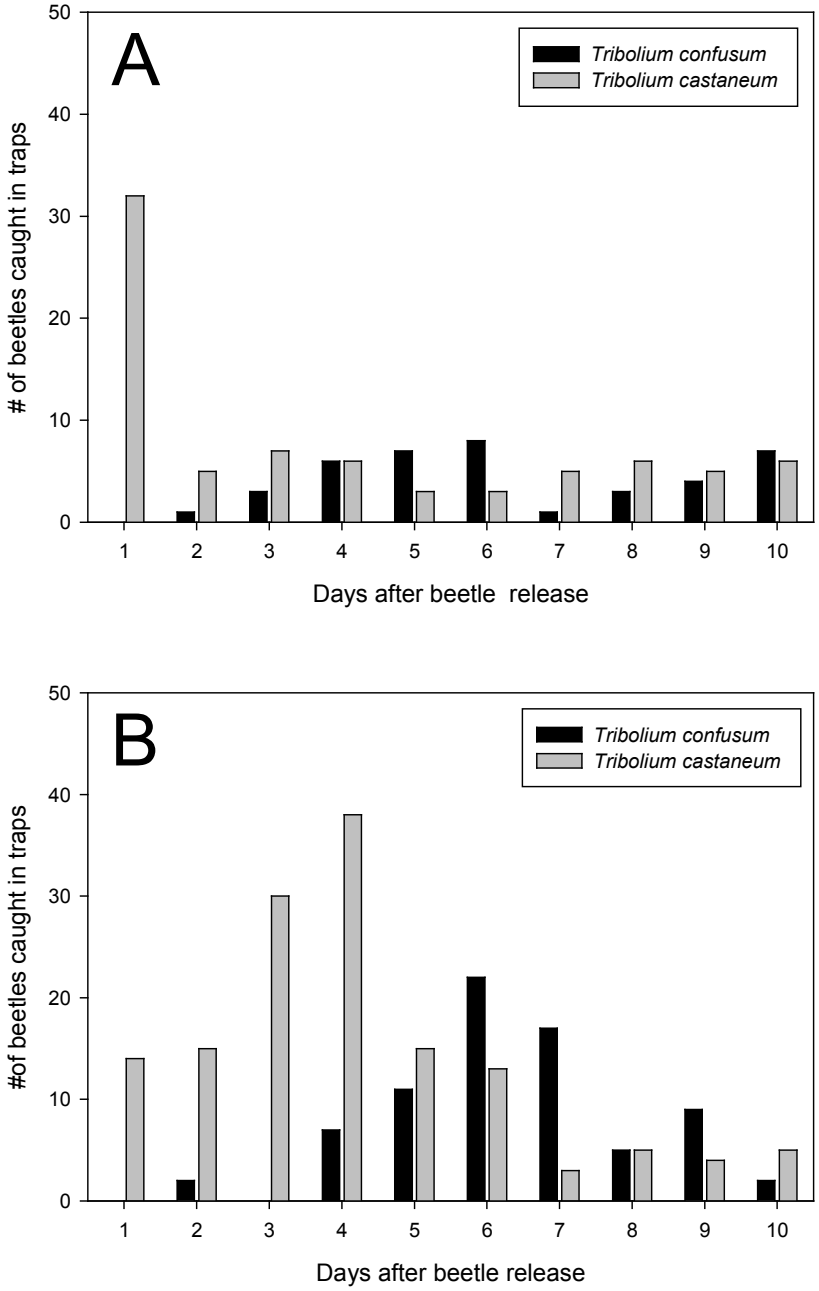

Figure 5.22. The number of beetles caught in pheromone traps over a 10-day period when 500 *Tribolium confusum* and 500 *Tribolium castaneum* beetles from laboratory cultures were released into a warehouse (16.1m×5.2m). A: trial 1, 7-17 March 2007; B: 6-16 January 2008.

Trials 1 for both mill beetles and laboratory beetles were carried out within a 6-week period by the same person, and trials 2 for mill beetles for laboratory beetles were carried out within a 5-week period by the same person. Because of this, comparisons were run comparing trial 1 laboratory/mill release, and trial 2 laboratory/mill release. More laboratory than mill beetles were caught in trial 1 when the numbers were compared for each species individually; see Figure 5.23. (*T. confusum:* $z=6.3$, $p<0.01$ using Bonferroni's inequality, Yate's correction used; *T. castaneum* : $z=3.4$, $p<0.01$ using Bonferroni's inequality, Yate's correction used). The same results were found with trial 2 (*T. confusum*: $z=7.9$, $p<0.01$ using Bonferroni's inequality, Yate's correction used; *T. castaneum*: $z=8.9$, $p<0.01$ using Bonferroni's inequality, Yate's correction used.)

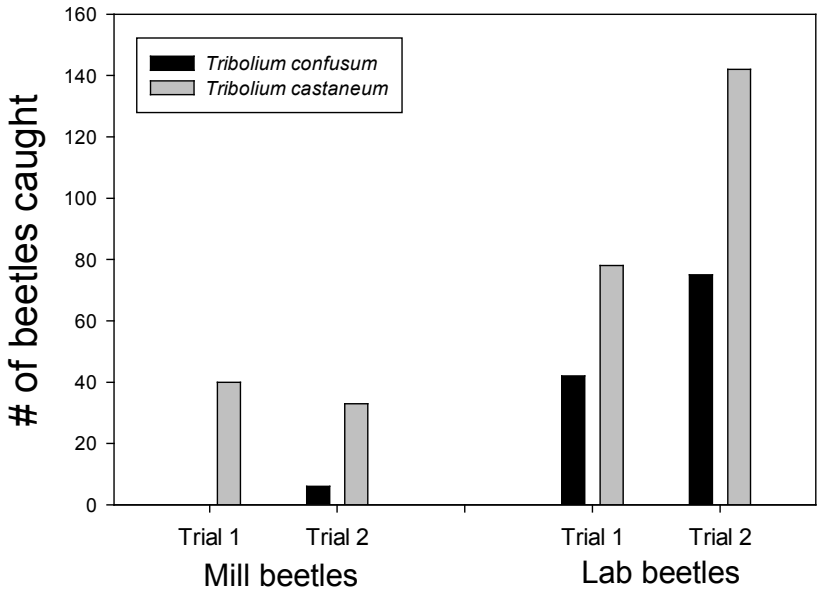

Figure 5.23. The total number of beetles caught during four recapture experiments. In each experiment, 500 *Tribolium confusum* and 500 *Tribolium castaneum* beetles (mill/laboratory strains) were released into a warehouse (16.1m×5.2m) containing 6 pheromone traps and traps were checked daily for 10 days after release.

Arena experiment

In trial 1 (baited traps), there was no significant difference, in terms of the number of beetles caught, between strains (two-way ANOVA, $F=2.8$, $p=0.11$) or species ($F=0.5$, $p=0.50$) but there was an interaction between species and strain ($F=5.85$, $p=0.03$). Because of this interaction, a one-way ANOVA was performed [log (x+1) transformation for normality] comparing the six separate groups (Mill 1-3 *T. castaneum*, laboratory *T. castaneum*, laboratory *T. confusum*, Mill 1 *T. confusum*). This revealed differences between the groups ($F=3.1$, $p=0.04$). Laboratory *T. castaneum* were caught more than any other group, *T. castaneum* from Mill 1 were caught the least and all other groups were mid-range [Tukey honestly significant difference (HSD) test; Table 5.5].

The number of beetles under the trap was counted for the last 3 out of 4 repetitions of trial 1. There were more *T. castaneum* under the trap than *T. confusum* (two-way ANOVA; $F=42.2$, $p<0.001$) but there was no difference between strains ($F=0.2$, $p=0.63$). There were differences between the groups (one-way ANOVA, $F=10.6$, $p<0.001$; Table 5.6). In trial 2 (baited traps), *T. castaneum* was caught more often than *T. confusum* [two-way ANOVA, log (x+1) transformation, $F=22.4$, $p<0.001$], laboratory beetles were caught more often than mill beetles ($F=4.8$, $p=0.05$) and there was no interaction between species and strain ($F=0.53$, $p=0.48$). A one-way ANOVA [log (x+1) transformation] revealed differences between the groups ($F=8.3$, $p=0.001$). Laboratory *T. castaneum* were caught more often than any other group, *T. confusum* from Mill 1 were caught the least and the other groups fell in the middle (Tukey HSD test; see Table 5.5).

In terms of the number of beetles under the trap, there were no differences for trial 2 between strains (two-way ANOVA, square root transformation, $F=1.9$, $p=0.19$) and species ($F=2.7$, $p=0.12$). More *T. castaneum* beetles from Mill 3 were under the trap than in the other groups and laboratory *T. confusum* and *T. castaneum* beetles were under the trap the least (Tukey HSD test; Table 5.6). Beetles under the trap were counted for all three repetitions.

For trial 3 (unbaited traps), there was no difference between species [two-way ANOVA, log (x+1) transformation, $F=0.03$, $p=0.59$], laboratory beetles were caught more often than mill beetles ($F=15.7$, $p=0.001$) and there was a significant interaction between species and strain ($F=6.1$, $p=0.03$). One-way ANOVA showed differences between the groups ($F=17.4$, $p<0.001$). Laboratory *T. castaneum* were caught the most, with the other groups caught significantly less than laboratory *T. castaneum* but not significantly more or less often than each other (Tukey HSD test; see Table 5.5).

Two-way ANOVA [log (x+1) transformation] showed that *T. castaneum* were found under the trap more often than *T. confusum* for trial 3 ($F=7.0$, $p=0.02$), with no difference between strains ($F=0.14$, $p=0.71$) or interaction between species/strain ($F=2.2$, $p=0.16$). There were differences between groups [one-way ANOVA, log (x+1) transformation, $F=4.8$, $p=0.01$]. For specific differences between the groups as revealed by Tukey HSD test, see Table 5.6. Beetles under the trap were counted for all three repetitions.

To isolate the effect of pheromone/oil, the results from trial 2 (baited traps) were compared to trial 3 (unbaited traps). A three-way ANOVA [log (x+1) transformation]

comparing strains, species and number of beetles in baited versus unbaited traps showed that baited traps caught more beetles than unbaited ($F=19.0$, $p<0.001$) but there were significant interactions for baited/unbaited versus species ($F=9.5$, $p=0.005$), and strain versus species ($F=4.9$, $p=0.04$).

Table 5.5. The number of beetles caught in a monitoring trap after 24-hours in dark conditions. Two hundred beetles were released into each of six arenas (1.2m×1.2m), with a trap in the middle, one species/strain per arena. Traps either contained pheromone and oil or no pheromone/no oil. Numbers reported are mean # of beetles in trap ± SEM.

Treatment	Trial	Number of beetles caught					
		Laboratory		Mill			
				1		2	3
		T. confusum	*T. castaneum*	*T. confusum*	*T. castaneum*	*T. castaneum*	*T. castaneum*
Pheromone/oil	1¶	18.3±8.3ab	27.5±5.8a	22.3±10.3ab	2.5±1.0b	8.8±3.1ab	5.5±1.9ab
	2*	2.3±0.9bc	35.3±15.7a	1.3±1.3c	3.3±1.2bc	17.3±5.8ab	9.3±1.5abc
No pheromone/ no oil	1*	27.0±9.5b	77.0±8.5a	15.7±6.7b	2.3±0.7b	12.0±4.9b	9.3±4.8b

¶ = 4 repetitions
* = 3 repetitions

For a given row, values denoted by different letters are not significantly different from one another ($p>0.05$).

Table 5.6. The number of beetles under a monitoring trap after 24-hours in dark conditions. Two hundred beetles were released into each of six arenas (1.2m×1.2m), with a trap in the middle, one species/strain per arena. Traps either contained pheromone and oil or no pheromone/no oil. Numbers reported are mean # of beetles under trap ± SEM.

Treatment	Trial	Number of beetles under trap					
		Laboratory		Mill			
				1		2	3
		T. confusum	*T. castaneum*	*T. confusum*	*T. castaneum*	*T. castaneum*	*T. castaneum*
Pheromone/oil	1	4.0±2.1bc	95.0±17.2a	9.3±5.6b	64.7±10.7ab	70.7±17.4a	97.7±14.3a
	2	0.0±0.0b	1.0±1.0b	0.3±0.3ab	9.3±3.3ab	2.3±2.3ab	19.7±12.9a
No pheromone/ no oil	1	1.7±1.7b	3.0±1.5ab	0.0±0.0b	4.3±2.3ab	16.0±12.5ab	27.3±11.3a

There were three repetitions in each case. For each row, values denoted by different letters are not significantly different from one another ($p>0.05$).

Movement rates - mill and laboratory beetles

Beetles taken from laboratory cultures moved faster than beetles directly from a mill (three-way ANOVA, $F=5.4$, $p=0.026$; Figure 5.24). There was no difference between the species ($F=1.3$, $p=0.27$) and no significant interactions between species, sex and strain ($p>0.05$ in all cases). One-way ANOVA showed a significant difference between different strains, species and sexes ($F=2.3$, $p=0.05$; Table 5.7 shows sample sizes). Male laboratory *T. confusum* moved the fastest, female mill *T. castaneum* moved the slowest and all other groups fell in the middle when compared using Tukey tests (Figure 5.24).

Laboratory beetles weighed more than mill beetles (three-way ANOVA, $F=4.3$, $p=0.044$), *T. confusum* weighed more than *T. castaneum* ($F=28.2$, $p<0.001$) and females weighed more than males ($F=10.1$, $p=0.003$). There were significant interactions between strain/species ($F=4.8$, $p=0.03$) and species/sex ($F=5.3$, $p=0.026$). One-way ANOVA showed a difference between the different strains, species and sexes ($F=7.7$, $p<0.001$). Female laboratory *T. confusum* weighed the most, with no significant differences between the other groups according to Tukey tests (Figure 5.24; Table 5.7). There was no significant correlation between overall movement rates and weights ($r^2=0.064$, $p=0.07$; Figure 5.26).

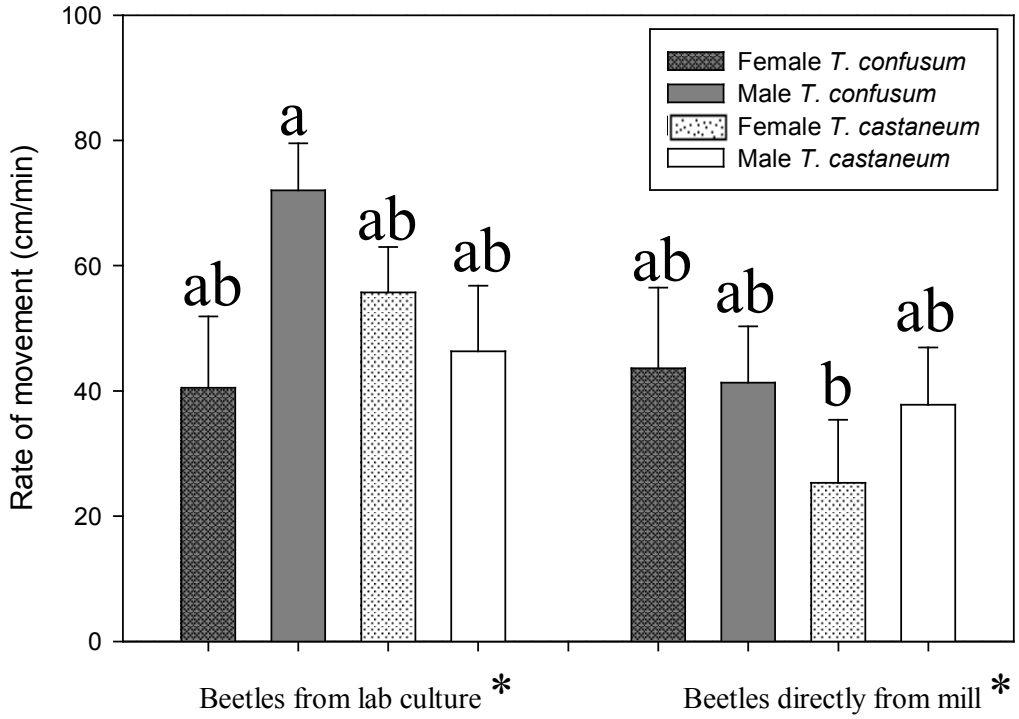

Figure 5.24. Movement rates for *Tribolium confusum* and *Tribolium castaneum*, taken directly from either a Canadian flour mill or from existing laboratory cultures. Bars with the same letters are not significantly different from each other ($p>0.05$).
* $F=5.4$, $p=0.03$

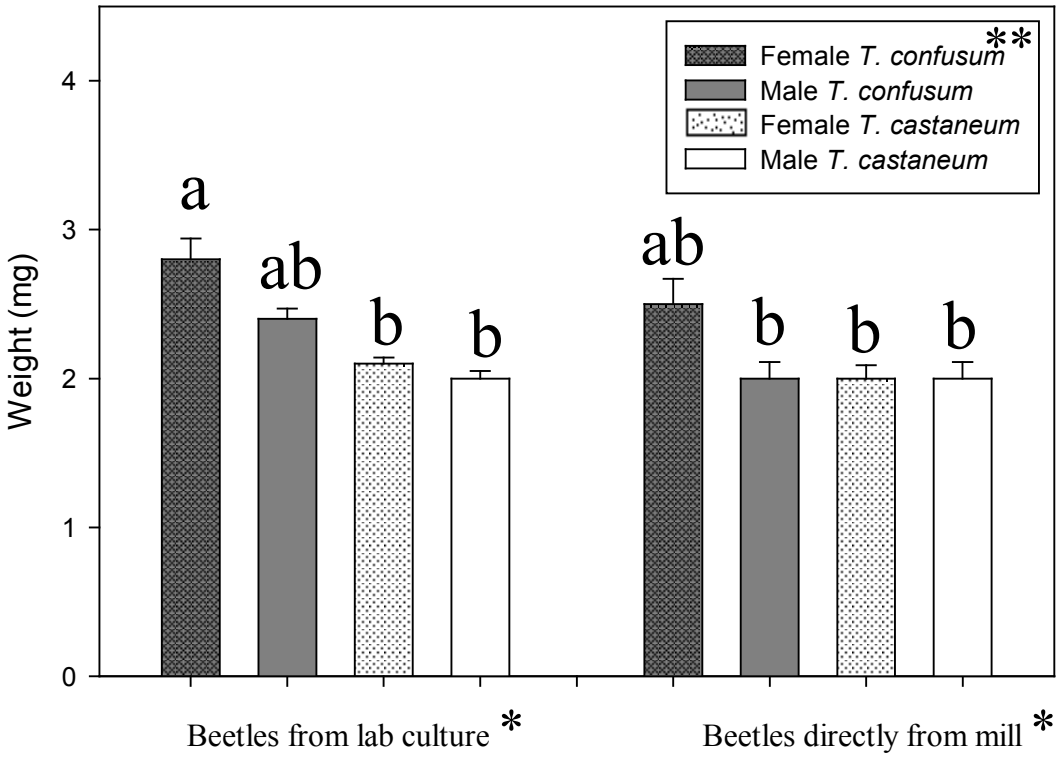

Figure 5.25. Weights of *Tribolium confusum* and *Tribolium castaneum*, taken directly from either a Canadian flour mill or from existing laboratory cultures. Bars with the same letters are not significantly different from each other ($p<0.05$).

* $F=4.3$, $p=0.044$

** females/males: $F=10.1$, $p=0.003$; *T. confusum/T. castaneum*: $F=28.2$, $p<0.001$; interactions: strain/species: $F=4.8$, $p=0.03$; species/sex: $F=5.3$, $p=0.026$

Table 5.7. Sample sizes for movement rate study of *Tribolium confusum* and *Tribolium castaneum*. Beetles were taken from mill or laboratory cultures, and reared either in a mill or in the laboratory.

			Tribolium confusum		*Tribolium castaneum*	
	Rearing	Strain	Female	Male	Female	Male
First experiment	Lab	Lab	6	7	9	4
(rearing different)	Mill	Mill	5	8	8	4
Second experiment	Lab	Lab	14	13	13	14
(rearing same)	Lab	Mill	15	15	13	14

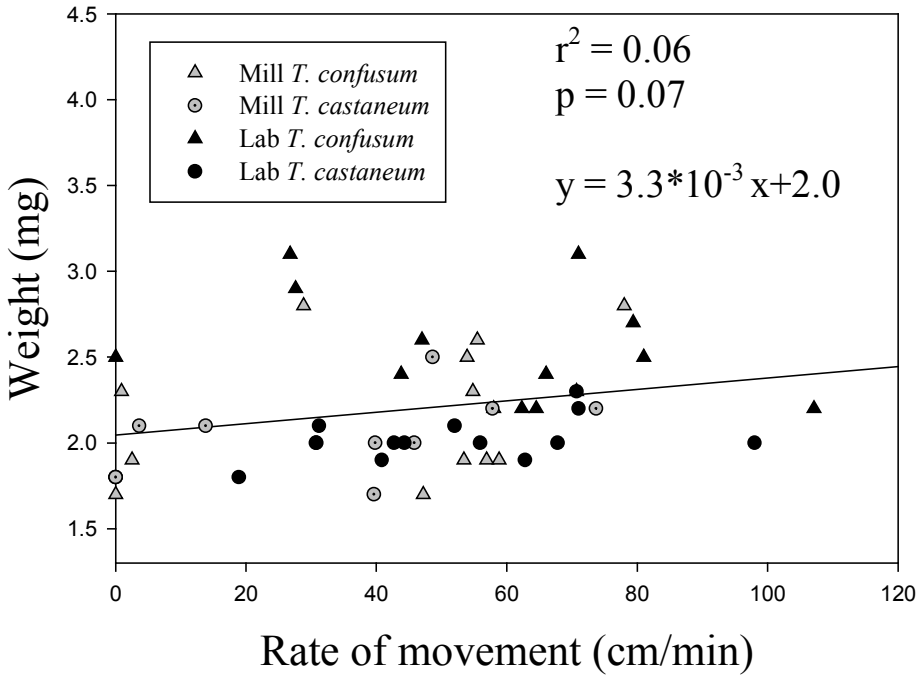

Figure 5.26. Correlation between weight and movement rates for *Tribolium confusum* and *Tribolium castaneum*. Beetles were taken directly from either a Canadian mill or from existing laboratory cultures.

When beetles were reared in the laboratory under identical conditions, there was no difference in movement rates between mill and laboratory strains (three-way ANOVA, $F=1.7$, $p=0.20$) but *T. confusum* moved faster than *T. castaneum* ($F=11.0$, $p=0.001$; Figure 5.27). No significant interactions between sex, species and strain were found ($p>0.05$). The three-way ANOVA on movement failed normality testing however, as did a one-way ANOVA comparing mill/laboratory, *T. confusum*/*T. castaneum* and male/female ($p<0.05$). A one-way ANOVA on Ranks showed a significant difference between different groups ($H=18.9$, $p=0.009$; Table 5.7 shows sample sizes). Once again, male laboratory *T. confusum* were fastest, female mill *T. castaneum* were slowest and the other groups were not significantly different from any group when compared using Dunn's method (Figure 5.27).

In terms of weight, significant differences were found between species (three-way ANOVA, $F=56.9$, $p<0.001$), strain ($F=11.0$, $p=0.001$) and sex ($F=29.5$, $p<0.001$) with significant interactions between species/strain ($F=28.7$, $p<0.001$) and species/sex ($F=14.1$, $p<0.001$). Using Tukey's HSD test, female laboratory *T. confusum* weighed the most of any group, female mill *T. confusum* and male laboratory *T. confusum* were second-highest in weight and the other groups weighed the least with no significant differences between them (Figure 5.28; Table 5.7 shows sample sizes). Overall, beetles in the second experiment tended to be heavier than in the first. There was a significant correlation between rate of movement and weight ($r^2=0.488$, $p=0.01$; Figure 5.29).

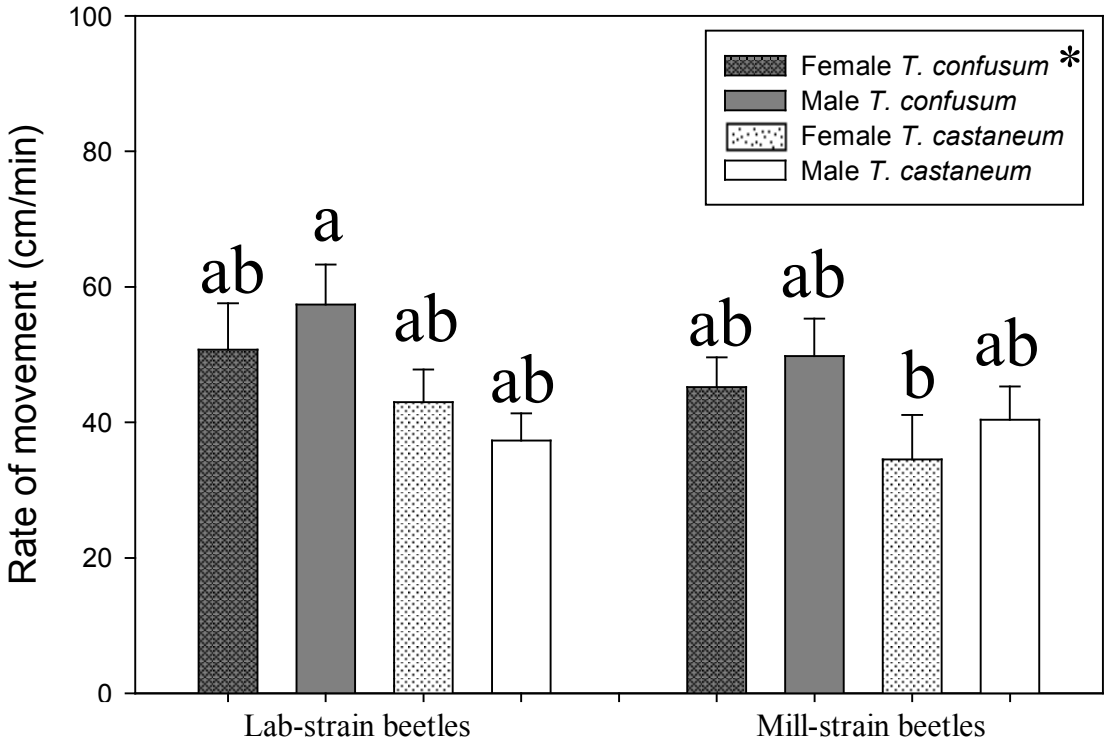

Figure 5.27. Movement rates for flour beetles, *Tribolium confusum* and *Tribolium castaneum*, taken from either mill or laboratory strains and all reared in the laboratory. Bars with the same letters are not significantly different from each other ($p>0.05$).

* *T. confusum/T. castaneum*: $F=11.0$, $p=0.001$.

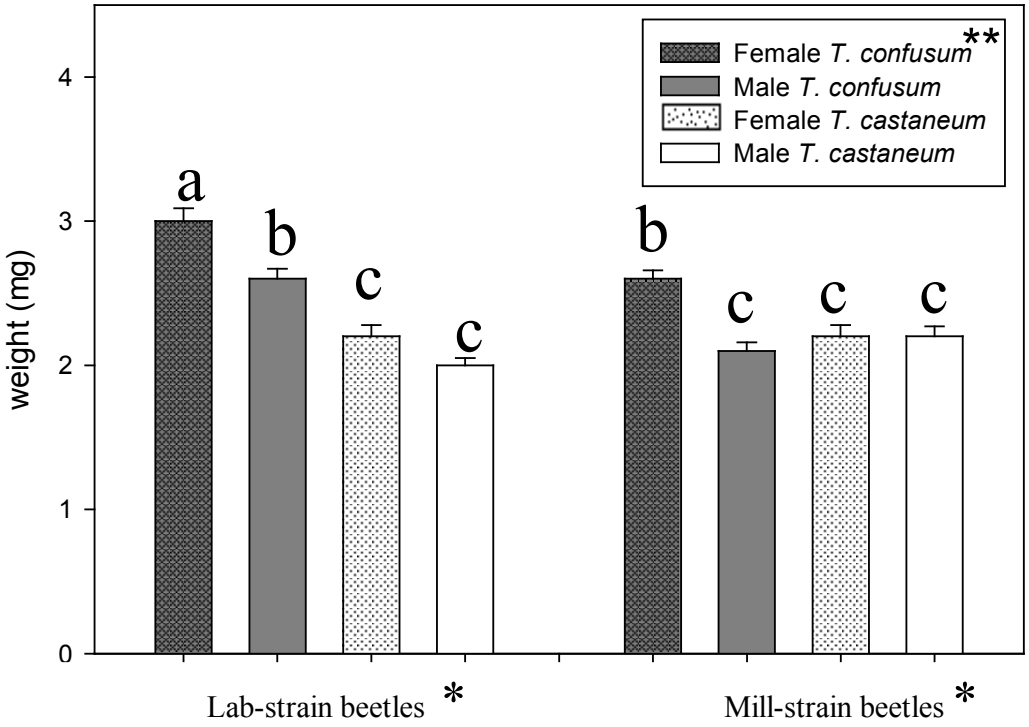

Figure 5.28. Weights of *Tribolium confusum* and *Tribolium castaneum*, taken from either mill or laboratory strains and all reared in the laboratory. Bars with the same letters are not significantly different from each other ($p<0.05$).

* $F=11.0$, $p=0.001$
** *T. confusum/T. castaneum*: $F=56.9$, $p<0.001$, female/male: $F=29.5$, $p<0.001$
Interactions: species/strain: $F=28.7$, $p<0.001$; species/sex: $F=14.1$, $p<0.001$

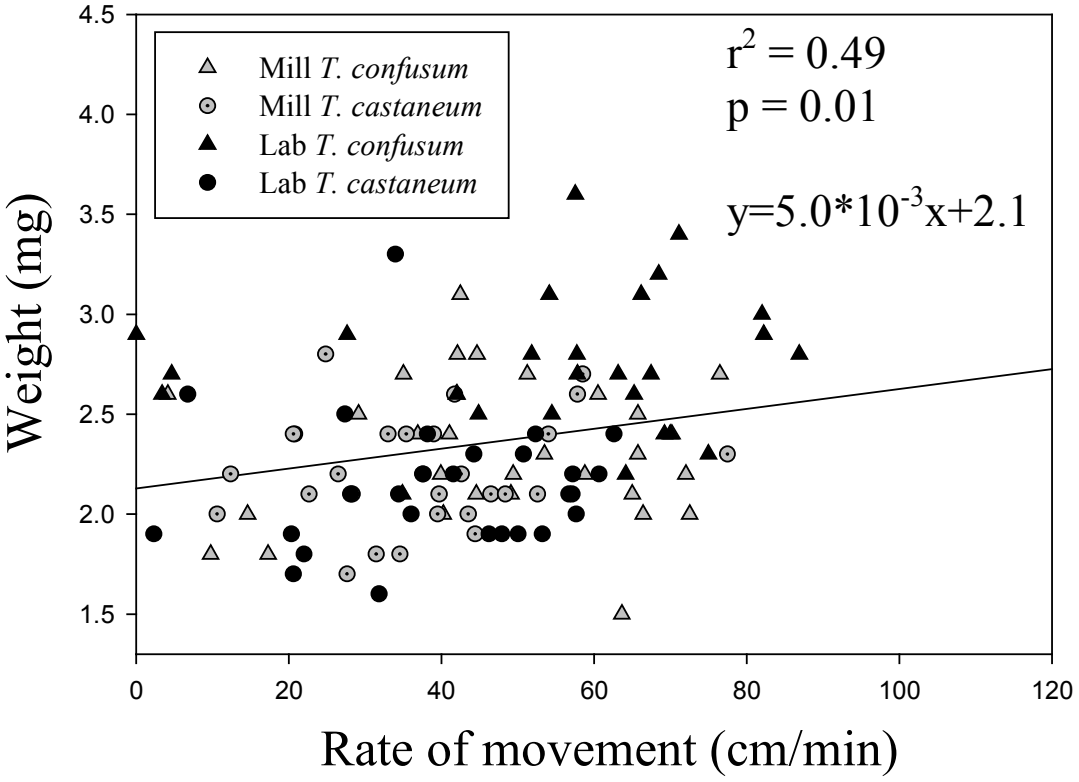

Figure 5.29. Correlation between weight and movement rates for *Tribolium confusum* and *Tribolium castaneum*. Beetles were taken from mill or laboratory strains and all were reared in the laboratory.

Comparing the two experiments, movement rates and weights were more variable in the first experiment than the second (Figures 5.24, 5.25, 5.27, 5.28). There were also some mill *T. confusum*, mill *T. castaneum* and laboratory *T. confusum* beetles that did not move at all in the first experiment (0 cm/min), while laboratory *T. confusum* was the only beetles that occasionally did not move in the second experiment.

Burrowing - mill and laboratory beetles

Beetle distributions from each of six groups (Mills 1-3 *T. castaneum*, Mill 1 *T. confusum,* laboratory *T. confusum* and laboratory *T. castaneum*) were compared using a generalized linear model procedure (Proc GENMOD). There were four repetitions for each beetle group, except Mill 3 *T. castaneum* which had three repetitions. (Over 5% of Mill 3 *T. castaneum* escaped from the tube during the fourth repetition so it was excluded.) All comparisons were significant ($p<0.05$) except when *T. castaneum* from Mill 2 and 3 were compared ($p=0.44$; Table 5.8). *Tribolium confusum* burrowed less than *T. castaneum* and laboratory strain beetles burrowed less than mill beetles (Figures 5.30 and 5.31). As well, *T. castaneum* beetles from Mill 1 burrowed less than *T. castaneum* from Mills 2 and 3 (Figure 5.32).

Table 5.8. Contrasts of beetle distributions, using a generalized linear model procedure. Beetles were placed in a column of flour for 24 hours and their distribution in the flour was then measured.

Beetle group	Comparison beetle group	Chi-square value	P value
Laboratory *T. castaneum*	Laboratory *T. confusum*	598.4	<0.0001
	Mill #1 *T. castaneum*	58.8	<0.0001
	Mill #2 *T. castaneum*	22.3	<0.0001
	Mill #3 *T. castaneum*	14.9	0.0001
Laboratory *T. confusum*	Mill #1 *T. confusum*	14.2	0.0002
Mill #1 *T. castaneum*	Mill #1 *T. confusum*	197.2	<0.0001
	Mill #2 *T. castaneum*	150.4	<0.0001
	Mill #3 *T. castaneum*	127.2	<0.0001
Mill #2 *T. castaneum*	Mill #3 *T. castaneum*	0.61	0.44

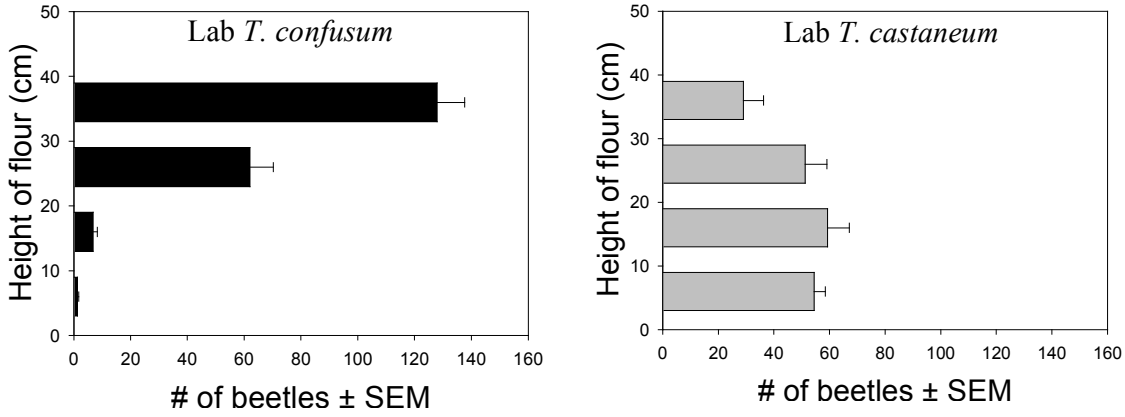

Figure 5.30. Distribution of laboratory *Tribolium confusum* and *Tribolium castaneum* in a column of flour after 24 hours. Distributions are significantly different from each other ($p<0.0001$).

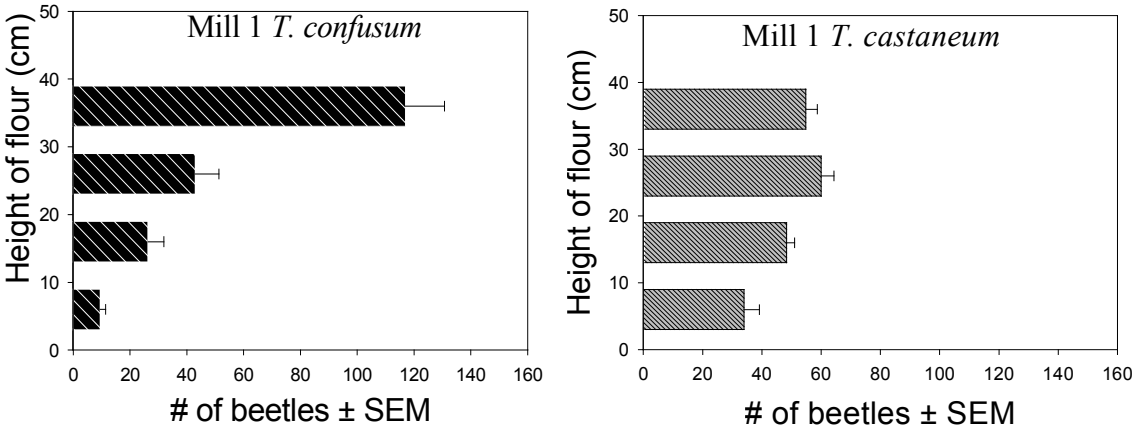

Figure 5.31. Distribution of Mill 1 *Tribolium castaneum* and *Tribolium confusum* in a column of flour after 24 hours. Distributions are significantly different from each other ($p<0.0001$).

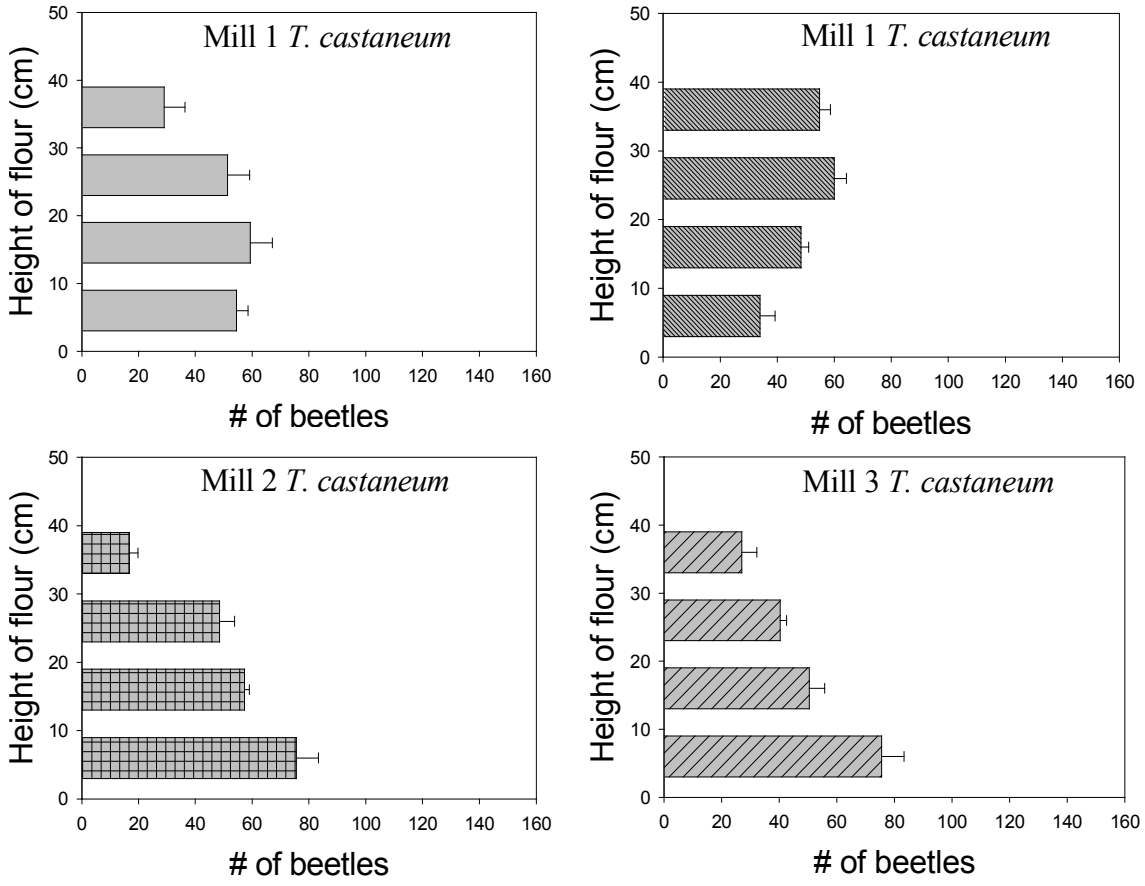

Figure 5.32. Distribution of laboratory *Tribolium castaneum* and Mill 1-3 *Tribolium castaneum* in a column of flour after 24 hours. All distributions are significantly different from each other ($p<0.0001$), except for Mill 2 *Tribolium castaneum* versus Mill 3 *Tribolium castaneum* ($p=0.44$).

Discussion

Pheromone traps showed low efficacy in the mill; less than 2% of *T. confusum* beetles released less than 60 cm away from a pheromone trap were caught. Brand new lures in traps can be repellent to *Tribolium* species (Hussain et al., 1994b). In this study, both a brand new lure and a 5-day-old lure did not differ appreciably. It is possible that

five days was not long enough to age the lure, but this is unlikely given that the traps reach peak effectiveness at one week (Hussain et al., 1994b) and that the trap had caught beetles on the floor of the mill prior to the experiment. The monitoring traps in this study are far less attractive than many other stored product traps. Pheromone traps for Indian meal moths and cigarette beetles are attractive enough to control populations through "mass trapping" (Pierce, 1994; Phillips et al., 2000). It is unclear why the traps caught so few beetles; it could be that the aggregation pheromone itself may not be that attractive to beetles (Mullen, personal correspondence). However, the pheromone/oil combination in the traps is attractive to some degree since baited traps placed in the packing plant caught significantly more beetles than unbaited traps.

This study reports, both in a mill and in a warehouse, that *T. confusum* is caught in pheromone traps less often than *T. castaneum. Tribolium confusum* may be less attracted because of the isomeric mixture present in the synthetic pheromone. The monitoring traps contain a mixture of 4R-8R-(-) and 4R-8S-(+)-4,8-dimethyldecanal (Mullen et al., 1992). While both *T. confusum* and *T. castaneum* have aggregation pheromones consisting of such a mixture, the ratio of the mixtures may be different for both species because *T. confusum* is 10 fold less attracted to the 4R, 8S-(+) isomer than *T. castaneum* (Suzuki et al., 1984; Suzuki and Mori, 1983).

The warehouse experiments also demonstrate that mill-strain beetles are caught less in traps than lab-strain beetles. Different strains of *T. castaneum* react differently to natural and synthetic pheromone in two-choice pitfall bioassays. *Tribolium castaneum* female beetles from four different strains, all reared in the laboratory for at least 7 years, differed in their sensitivity to synthetic aggregation pheromone, with some of the beetles

preferring higher concentrations than others (Boake, 1984). Many of the populations also discriminated against the pheromones emitted by males from their own strains in favor of males from other strains, a possible method of reducing inbreeding among populations (Boake, 1984).

The results reported here have significant implications for mill trap monitoring. With a smaller proportion of *T. confusum* being caught in traps, mills infested with *T. confusum* may have higher levels of infestation than previously thought. Also, much of our knowledge on pheromone attractiveness and trap monitoring comes from lab-strain beetles, which do not behave like mill-strain beetles. More research is needed on the differences in trap efficacy between species and strains. More research also needs to be done to make these monitoring traps generally more attractive to beetles. Further research on the chemical components of *T. confusum* and *T. castaneum* aggregation pheromones would be useful, as would research on the effects of adding other food volatiles to the traps.

There were substantial differences between the arena results and the warehouse results/mill sample analysis. The differences between the arena and warehouse experiments were plentiful however. The warehouse experiment was designed to simulate mill conditions; the lights were left on and there were packages and tables and other items left in the warehouse. The arena experiment, on the other hand, had lights turned off and the arena was a simple and more confined environment. These beetles behave differently around borders and edges (Campbell and Hagstrum, 2002) and their movement is affected by light conditions (Amos and Waterhouse, 1967; Park, 1934; Amos, 1968). There was probably also small amounts of food available to beetles in the

warehouse while the arenas did not contain food (since they were vacuumed thoroughly between repetitions); food patches affect trap catches (Toews et al., 2005). An analysis of the warehouse experiments also show great variation in the number of beetles caught in traps daily (Figures 5.20 and 5.22); in trial 2 (laboratory beetles) for example, 3-38 *T. castaneum* and 0-22 *T. confusum* were caught depending on the sampling day. These traps may not be accurate enough for a 24-hour test.

Mixed-species cultures of *T. castaneum* and *T. confusum* cannot exist indefinitely in the laboratory, with one species eventually eliminating the other (Park, 1948; Park, 1954; Park, 1957). In contrast to this, the present study found a mix of *T. confusum* and *T. castaneum* in every flour sample taken from the mill. This species mix may occur because of a lack of competition. The beetles in Park's studies were reared in the laboratory under extremely high densities (100s of beetles in 8 g of flour) while beetles in mills exist under much lower densities. These two species compete with each other through cannibalism and cannibalism is a often an animal's response to high density (Fox, 1975).

The beetles in Park's studies co-existed for years before one species was victorious over the other; mill beetle populations are decimated regularly due to fumigations. That being said, there is some evidence that *T. confusum* and *T. castaneum* existed together in this mill at least 5 months prior to sampling (Hawkin, unpublished data). The literature does not exclude the possibility of co-existence, even in laboratory settings; Leslie et al., (1968) reported a mixed-species culture of *T. confusum* and *T. castaneum* that had lasted up to the end of the study (960 days). Still, such a mix is rare; Trematerra and Sciarretta (2004) note that populations of *T. confusum* and *T. castaneum*

seen in a feed mill rarely overlapped and when they did one of the species rapidly dispersed away from the other. Whether these two species co-exist in other mills as they do in the mill studied here remains to be seen.

The food available to mill beetles is less nutritious than that given to our laboratory beetles. [See Smallman and Loschiavo (1952) for a nutritional analysis of flour at different stages of milling.] Beetles in our laboratory are reared in medium containing brewer's yeast, an ingredient that improves the productivity of both *T. confusum* and *T. castaneum* (Sokoloff, 1974). Beetles in mills may also have less access to food than laboratory beetles, which are given an unlimited food supply. The difference in food quality and quantity may explain why mill beetles weigh less than laboratory beetles. Mills are also dangerous for beetles; many of the mill beetles brought back to the laboratory were missing legs and antenna. It is possible that the beetles directly from the mill moved slower than laboratory beetles because they were injured in an unobvious manner. While some strains of *Tribolium castaneum* move slower than others (Rigaux et al., 2001), the different strains we tested the beetles in this study did not move at different rates. Still, selective pressures on mill and laboratory beetles could eventually result in differences that cannot be seen after one generation.

Mill beetles are genetically different than laboratory beetles in some ways; the mill-strain beetles weighed less than laboratory beetles, mill beetles were caught less in traps and mill/laboratory strains dispersed differently in a flour mass. The laboratory stocks used were decades old, enough time to produce hundreds of generations. *Tribolium confusum* and *T. castaneum* have different numbers of larval instars depending on conditions (Sokoloff, 1972). Since laboratory food is more nutritious for beetles than

mill food, generations of laboratory beetles may consistently undergo more larval instars, resulting in heavier beetles.

Laboratory beetles are reared under much higher densities than mill beetles, so selective pressures may explain the differences between mill and laboratory beetle burrowing tendencies. Density has a negative effect on both species: *T. confusum* and *T. castaneum* disperse more as interspecific populations increase (Naylor, 1959; Naylor, 1961). Generations of high-density rearing would result in generations of beetles that do not burrow but rather try to disperse from the flour source. The way that beetles are cultured may affect burrowing; 100-200 beetles are typically taken from filter paper sticking out on top of the culture. *Tribolium confusum* is also repelled by fresh flour, dispersing from the surface of fresh flour more than conditioned flour, i.e. flour that has been previously exploited by *Tribolium* and contains quinones (Naylor, 1959; Ogden, 1970). Being reared under repelling, high-density conditions could result in beetles that barely burrow at all, i.e. the laboratory strain of *T. confusum* used in this study. While *T. confusum* is repelled by fresh flour, *T. castaneum* prefers fresh flour to conditioned (Sokoloff, 1974), resulting in a tendency to burrow more.

Mill *T. confusum* burrowed more than laboratory *T. confusum* in this study; this may be because these mill strain from the mill had not been reared under laboratory conditions for as long as their laboratory counterparts. There is a genetic (i.e. heritable) component to burrowing however, since Mill 2 and 3 *T. castaneum* showed different distributions than Mill 1. This is not surprising since there are genetic components to other *Tribolium* behaviours, i.e. movement (Rigaux et al., 2001) and cannibalism rates (Park et al., 1964).

This chapter has outlined many differences between species and strains of *Tribolium*. The differences shown could affect trap catches in ways not seen here. Mill-strain beetles are less attracted to traps when released in a warehouse lacking harbourage sites. Since mill beetles burrow more, they may be captured even less in mills. Beetles in mills may also be caught less than the mill-strains seen here because they move slower and therefore would be less likely to come in contact with traps. Since effective population sampling is a critical part of any IPM practice and behavioural differences can affect trap efficacy, further research into the behaviour of beetles in mills is needed.

Chapter 6: General Conclusions

In order to use IPM effectively, one must understand the biology and behaviour of stored product pests and how to best sample for their populations in food processing facilities. This concluding chapter will discuss the findings reported in this thesis in terms of their relevance to IPM. First, behavioural differences between mill/laboratory and *T. castaneum/T. castaneum* will be discussed. Second, trap efficacy will be examined.

Most behavioural studies on *T. castaneum* and *T. confusum* have used laboratory strains. Mill beetles behave differently than laboratory beetles however; mill beetles burrow more than laboratory beetles and beetles directly from a mill move slower than laboratory beetles (Chapter 5). These behavioural differences may impact one's ability to spot beetle activity during visual inspections in mills. Since mill beetles burrow more, they may be more likely to inhabit hidden areas like equipment and cracks and less likely to be spotted on the floor of a mill. Mill beetles may also leave behind fewer tracks on a mill floor, since they burrow more and move slower. In order to detect low-level populations in mills, it is suggested one uses more vigorous visual inspection methods such as regularly sampling deep within equipment and closely examining any cracks for signs of insect presence.

This thesis contains the first report of extensively-mixed populations of *T. confusum* and *T. castaneum* in a mill. The frequency of such mixed populations in other mills is unknown. Trematerra and Sciarretta (2004) report both *T. confusum* and *T. castaneum* present in a mill but little, if any, overlap of the two species. However, studies on species distributions in food processing facilities tend to rely on traps and bait bags to gather data (Campbell and Arbogast, 2004; Trematerra and Sciarretta, 2004), rather than

examining the species present within flour samples. Such sampling methods may be biased towards *T. castaneum.* This thesis has shown that pheromone traps catch more *T. castaneum* than *T. confusum* (Chapter 5). The bait bags used in Trematerra and Sciarretta (2004) contained wheat, broken carobs, broken peanuts, kibbled oats, broken almonds, raisins, dried bananas and broad beans. Since *T. confusum* is primarily restricted to flour mills while *T. castaneum* can be found in flour mills and grain processing facilities, perhaps such a food combination is more attractive to *T. castaneum* than *T. confusum.* While Campbell and Arbogast (2004) also took some samples from the product stream and trash and analysed the beetles within, there were many more samples taken and analysed in this thesis, and the samples were generally larger and taken from more areas of the mill. More work on mixed populations, using extensive flour sampling rather than trap or bait bag data, needs to be done.

Even though this thesis reports mixed populations in a mill, almost exclusively *T. castaneum* populations were found in rollstand samples taken from the same mill less than a year after mixed populations were found. This may not indicate a lack of species mix overall in the mill, rather it may simply indicate a lack of mix in the rollstands. *Tribolium confusum* may be more likely to inhabit easy-to-access areas of a mill rather than equipment interiors because it cannot fly (Sokoloff, 1972) and it is less likely to exploit new territories than *T. castaneum* (Ziegler, 1976). Further studies on species mix in mills should analyse samples from as many areas of the mill as possible, from within equipment and from more easily accessible areas. Through extensive sampling of a variety of mill areas, more species mixes may be found in mills.

Pheromone traps show low efficacy in catching *Tribolium* spp., both in mill and laboratory settings (Chapter 5). There are many possible explanations for this. Beetles touch the trap more often than they enter it (Chapter 5) so the trap design may be problematic. The texture and incline of the ramp may deter beetles. Alternatively, the trap design may interfere with the behavioural response to the pheromone, which includes extention of the prothoracic leg extension, antennal protraction and zig-zag movement towards the pheromone source (Faustini et al., 1982); it may be difficult for a beetle to zig-zag onto and up the ramp for example. Further research into beetle behaviour around and under these traps is needed.

The traps' low efficacy may be a result of the low attractiveness of the pheromone itself (Mullen, personal communication). This may be because the natural aggregation pheromone is not very attractive, or it may be a problem with the synthetic aggregation pheromone used in the traps. A two-choice pitfall bioassay study with both species, comparing the attractiveness of trap pheromone lures to natural pheromone, would be useful. There could also be a problem with the airborne release of the pheromone in traps. As Boake (1984) points out, *T. castaneum* spends its life in flour so it may not normally react to airborne chemicals. Future work needs to be done to see if this is the case. If it is, perhaps another trapping method entirely should be discussed.

This thesis' report of strain and species differences in trap efficacy is the first of its kind. Laboratory beetles are reared in the presence of higher levels of aggregation pheromone than mill beetles, because they are raised under higher densities. Hussain et al., (1994b) report than laboratory *T. castaneum* are repelled by pheromone traps with lure that are less than one week old because the pheromone levels are too high; perhaps

mill beetles are repelled at even lower doses because of their lack of previous pheromone exposure, and therefore lures in mills need to be aged more for peak effectiveness. More research on mill beetles and their attraction to traps needs to be done to see if this is the case.

Tribolium confusum may be less attracted to traps than *T. castaneum* because of the pheromone used in these traps. It is suspected that both species have similar aggregation pheromones consisting in part of 4R-8R-(-) and 4R-8S-(+)-4,8-dimethyldecanal, but the ratios of the two isomers may be different for each species (Suzuki et al., 1984). There are also probably additional components to the pheromone (Boake, 1984). More work on *T. castaneum* and *T. confusum* pheromone biology is needed.

The low efficacy of these traps and the differences in trap attractiveness between strains and species currently make these traps useful for qualitative, not quantitative assessments. The traps will be more useful for IPM if they are sensitive enough to detect small populations and small changes in beetle population sizes. The traps also need to be more attractive to beetles if they are to ever be used to create economic thresholds. There are several areas that should be more thoroughly investigated in order to make these traps more sensitive. Some of these have been mentioned above: more work on *T. confusum* pheromone biology is needed, along with studies on beetle behaviour in/around traps. There should also be more research into the unidentified pheromone in the frass of *T. castaneum* (Suzuki, 1985); perhaps adding a synthetic version of this pheromone to traps may increase the number of *T. castaneum* caught. More work on food volatiles is needed, since adding such volatiles may help increase efficacy for both species. Most importantly,

there needs to be more research into how mill beetles behave in mills and around pheromone traps. Only a thorough understanding of the behavioural differences between mill and laboratory beetles will help in the translation of laboratory research into useful IPM practices in mills.

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