Insect population distribution and density estimates in a large rice mill in Portugal – a pilot study

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Highlights

- *Sitophilus zeamais* Motschulsky is the key-pest of stored rice followed by *S. oryzae* (L.).
- *Sitophilus* spp have an aggregated dispersion pattern and surpassed the milling process.
- From Green's sampling plan and RVSP, our corrent sample size would yield a precision level of 0.30.
- For a precision level of 0.25, the sampling plan should add eleven traps.
- This sequential sampling plan for *Sitophilus* spp. can be applied as IPM tactics at rice facilities.

Insect population distribution and density estimates in a large rice mill in Portugal - a pilot study.

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Abstract

The objectives of this study were to characterize the spatial distribution of the most abundant insect species identified in the rice plant, optimize the sampling program, develop and validate a fixed-precision enumerative sequential sampling plan for the key pests *Sitophilus* spp. in a rice industry in Portugal.

Experiments were carried out from September 2005 to July 2007, using 25 pitfall traps baited with food grade oil and pheromone specific for *Sitophilus* spp. The traps were observed weekly and the insects were identified and counted. Several species were found but *Sitophilus zeamais* Motschulsky and *S. oryzae* (L.) were the most abundant (90% of the total) followed by *Tribolium castaneum* (Herbst). Taylor's power law parameters, from the regression of log variance versus log mean, suggest an aggregated distribution for *Sitophilus* spp. and *T. castaneum*.

A fixed-precison sequential sampling plan was developed for *Sitophilus* spp., using Green's fixed precisions sampling plan and the Resampling Validation of Sampling Plan, with an action threshold of 0.5 *Sitophilus* spp. The sampling plan was designed to provide precision levels of 0.20, 0.25 (for pest management purposes), 0.30 and 0.35. The current sample size raises a precision of 0.30, and an increase of the number of traps to 37 would be recommendable to fit the desirable precision of 0.25. This fixed-precison sequential sampling plan for *Sitophilus* spp. populations in rice demonstrated to be useful as a tool in IPM tactics at rice facilities.

Key-words: stored rice, *Sitophilus* spp., *Tribolium castaneum*, floor traps, fixed precision sampling plan, distribution pattern, Taylor's power law.

1. Introduction

Portugal is a major rice consumer among the European Union countries. The annual per capita consumption of rice in Portugal is 15 kg, 2.5 times higher than in Spain, which is the second largest consumer of rice in the EU. About 2,000 rice farmers and eight rice processors in Portugal annually produce of 129,000 metric tons (MT) of *japonica* rice and 26,000 metric tons of *indica* rice, primarily in three main regions: Mondego (36,000 MT of *japonica* rice); Tejo (49,000 MT of *japonica* rice and 6,500 MT of *indica* rice) and Sado Valley (43,400 MT of *japonica* rice and 19,500 MT of *indica* rice). In Portugal rice is a seasonal crop; planting takes place in April and is harvested near the end of August. As a consequence, the maintenance of paddy and milled rice quality is very important for availability year-round. The demand by the consumer for better quality rice has increased, resulting in all aspects of rice production from breeding to agronomic practices to storage.

Paddy rice in storage is subject to infestation by stored-product insects which can contribute to qualitative and quantitative losses. While a a great amount of research has been conducted on insect pests in wheat storage (Smallman and Loschiavo, 1952; Subramanyam et al., 2005; Toews et al., 2006; Campbel et al., 2010a,b), little information is available on stored-product insects in facilities where rice is milled and packaged. Mills are ideal habitats for stored-product insect pests. These insects become of economic significance because of year-round warm temperatures and constant availability of food resources that allows for continuous damage (Smallman and Loschiavo, 1952). Under an IPM strategy, management of insects in mills is important in order to produce wholesome and unadulterated products (milled rice). Recommended IPM practices in mills include inbound inspection of raw materials (paddy rice), sanitation, exclusion, stock rotation, use of residual insecticides in non-food areas, and well-timed use of whole-facility treatments with heat or fumigants. Research in wheat flour mills has shown that managing insect infestations in mill environments is difficult because of their continuous operation (24 h a day 7 days a week); inadequate inbound inspection of materials, ineffective sanitation and exclusion practices (Subramanyam et al., 2005), and improper timing of pest management interventions (Toews et al., 2006). Insects also infest structures and equipment in areas that are difficult to access (Campbell et al., 2010ab) that can result in reservoirs of pests that can reinfest product after control measures have been implemented.

The rice industry in the European Union is currently facing serious problems related to insect contamination due to the restrictions placed on the use of chemical pesticides. Some grain protectants that were historically used on rice have been removed from the EU market due to insecticide resistance or through regulatory action. An example is the organophosphate insecticide malathion, once extensively used in pest management programs in stored grains, but has been largely discontinued in many countries. Although consumers express preferences for residue-free food products, the rice storage industry must occasionally use insecticides to maintain quality and prevent economic damage. In addition, in many countries insects are developing resistance to insecticides, including the fumigant phosphine (Price and Mill 1988, Chaudhry 1999, Zettler and Cuperus 1990). Thus, the implementation of an IPM program assists in minimizing the use of pesticides and targets when they are most needed.

The lack of information on insects in rice mills, especially in Portugal, prompted us to conduct a two-year study to understand the populations of insect species found and to determine the optimal number of traps (the "sample size") needed for the estimation of species density in this rice milling plantThis information can then be used to achieve effect control in the most efficient manner. The advantage of sequential sampling is to optimize sample size required for decision making and therefore saves time and money and minimizes the error of ineffective control measures. Therefore it is important to validate the sampling plan to determine actual error rates for IPM tactics, either in this facility or in other similar rice plants.

2. Materials and methods

2.1. Rice plant environment

This study was conducted in a 30 year old rice facility that belongs to a producer association in the Tejo Valley region of central Portugal. This particular facility has a storage capacity of 23,000 tons for paddy (using corrugated metal bins) and 1000 m^2 warehouse for storage packaged milled rice. The facility processes 17.500 MT of rice annually of varieties such as Ariete (*japonica*) and Jasmine (*indica*). Paddy rice received at the rice mill usually comes directly from the field at harvest and is dried to 13-14% moisture and stored in corrugated metal bins until processing occurs.

The rice processing plant structure has a total area of 2,000 m^2 divided into three main areas: reception, factory and packaging (Figure 1). The *reception area*, as indicates, is for rice receival, inspection and determination of quality attributes of the rice. The *factory* is where stored paddy rice is brought in, cleaned, processed and polished. This typically includes de-stoning machine, sifters, sieves for classifying contaminants, roller stands, brown rice separator, rice polishers, and processed white rice classifying sieves. In the *packaging area* the milled rice is packaged, labelled, and stacked onto pallets. This area also contains a laboratory where rice samples are examined and stored for final product quality purposes.

2.2. Insect sampling experimental design

Adult stages of insects associated with stored rice were sampled with commercial floor traps continuously from 9 September 2005 to 29 June 2007. From 9 September 2005 to 15 September 2006, 25 Stogard® Beetle Trap (Dome design) baited with food based oil, (Trécé Inc., Adair, OK, USA) were used to capture insects' species present and to monitor residual populations of the key pests. From 15 September 2006 to 29 June 2007, these traps were replaced by 25 PC Floor Traps baited with sitophilure, the synthetic *Sitophilus* spp male aggregation pheromone lure (Insects Ltd, Westfield, IN, USA).

The traps were placed at various positions around the rice mill and the majority of the these devices were located close to the machinery to follow the relationship between steps in rice processing and insect infestation (Figure 1). Traps were checked weekly and the insects collected were placed in a plastic bottle and brought to the laboratory for species identification and counting. Pheromone lures in traps were replaced monthly.

2.3. Characterizing spatial distribution of insects

Taylor’s power law regression was applied to each data set and used to classify the spatial distribution of *T. castaneum* and *Sitophilus* spp. in order to develop a sequential sampling plan for *Sitophilus* spp. To classify the spatial distribution of *T. castaneum* a total of 82 data sets were collected data was used in the Taylor’s model. To develop sequential sampling plans for *Sitophilus*, from a total of 86 data sets collected, two groups of counts were randomly selected: 71 data sets we used for estimation of the Taylor’s power law parameters, to classify the spatial pattern and to be used at Green’s plan; and 15 data sets for validation with Resampling for Validation of Sampling Plans (Naranjo and Hutchison, 1997).

Taylor’s power law is a regression used to model the relationship between mean ($\bar{x}$) and variance (s^2) and was applied for *Sitophilus* spp. and for *T. castaneum.* The most common relationship is: $s^2 = A\bar{x}^b$, where *b is* the slope and *A* was determined by taking the antilog of the intercept *a* from regression analysis. Sample means and sample variances are transformed using logarithmic equation. The intercept coefficient is a scaling factor related with sample size and *b* is a constant depending of insect’s behaviour: $b=1$, $b>1$ and $b<1$ indicates random, aggregated or uniform dispersions, respectively (Davis, 1994).

Once it is validated for a particular ecosystem, we can predict the variance of a set of counts, based on the known mean density, and design a sampling plan to obtain estimates of specified precision (Krebs,1999).

2.4 Green’s fixed precisions sampling plan for estimating density of Sitophilus spp..

Based on the previous results the next step was to choose the method to obtain an approach to specify boundaries for sample size and in our case an upper limit. Within this context, Green’s fixed-precision sampling plan was used, using the two parameters *a* and *b* of the Taylor’s power law regression for *Sitophilus* spp. (Taylor, 1961; Green, 1970; Naranjo and Hutchison, 1997).

Green (1970) developed an equation using Taylor’s *a* and *b* coefficients for estimating mean density sequentially with a fixed level of precision and defined the stop line by the log-regression:

$$\log_{10} T_n = [\log_{10}(d^2/a)/b-2) + (b-1)/b-2] * \log_{10} n$$

Where T_n is the total number of insects sampled, n the number of sample units and d is the precision (or one standard error according Krebs, 1999) expressed as the ratio of the standard error of the mean to the mean (SEM/mean) (Toews et al., 2003). The levels of precision decided were 0.20, 0.25 [for pest management purposes suggested by Southwood (1978)], 0.30 and 0.35.

2.5 Resampling Validation of Sampling Plan (RVSP)

RVSP randomly selects observations from an actual data set until either the sequential rule is satisfied or a fixed number of samples have been drawn, depending on the sample plan tested. This process is repeated numerous times (default=500) for each data set. Based on these iterations, RVSP then calculates averages and variances for precision and sample size, as well as operating characteristics for the binomial plans which classify population densities relative to a specified threshold. RVSP provides both summary and detailed output that can be easily imported in spreadsheet and graphical programs for further examination and analysis (RVSP, Naranjo and Hutchison, 1997).

To validate the sequential sampling plan for *Sitophilus* spp., 15 data sets were randomly selected from the estimation of the Taylor’s power law parameters and were used as independent Resampling Validation of Sampling Plan.

Simulations were used to randomly sample with replacement from each of the validation data sets until the stop lines were crossed, which generally indicates the desired precision has been achieved. After 500 interactions of sampling from each data set, the mean density, sample number and precision were obtained. The average across validation data sets of the resulting mean sample number for each of the precision levels are the recommended sample numbers for estimation of weevil density, according Koch et al (2006) and O’Rourke and Hutchison (2003). Using RVSP simulation software (Naranjo and Hotchison, 1997)] the resulting *A* and *b* values from Taylor´s power law were used to validate Green’s (1970) enumerative sampling plan with the precision levels of 0.20, 0.25, 0.30 and 0.35.

3. Results and discussion

3.1. Total number of insects caught

A total of 8963 insects were captured during experiments. *Sitophilus* spp. [*S. zeamais* Motschulsky and *S. oryzae* (L.)] were the most abundant in all areas of facility, comprising 90% of the total, with remainder of captures being *T. castaneum*, 8% of the total (Table 1).

The 2% of the insects captured belonged to other species as *Rhyzopertha dominica* (F.) (Coleoptera, Bostrychidae), *Gnathocerus cornutus* (F.) (Coleoptera, Tenebrionidae), *Cryptolestes ferrugineus* (Stephens) (Coleoptera, Laemophloeidae), *Typhaea stercorea* (L.) (Coleoptera, Mycetophagidae), *Cryptophagus* spp. (Coleoptera, Cryptophagidae), *Anisopteromalus calandrae* (Howard) (Hymenoptera, Pteromalidae), and predators from Carabidae family with a total of 181 individuals trapped.

A sub-sample of 231 adult weevils was taken to identify species using genitalia characters (Haines 1991). The maize weevil S. zeamais was the predominant species: 218 adults were *S. zeamais* (123 females and 95 males) and 13 were *S. oryzae* (eight males and five females). Many rice farmers also produce maize and use the same equipment for both grains, so it is possible that this is a mechanism for this species dominance on rice.

Considering that *Sitophilus* spp. and *T. castaneum* were well established it will be difficult to categorically document that the density of one beetle species is high or low due to the effect of another species, because of feed-back interactions between species (Nansen et al., 2009). The main reason for not including interspecific associations in models of stored-product pests have been that dynamics of beetles (Hagstrum and Milliken, 1988; Jacob and Fleming, 1989; Throne, 1994) populations are believed to be driven mainly by temperature and moisture content.

3.2. Relative abundance of *Sitophilus* spp. and *Tribolium castaneum* –relationships with environmental conditions

Inside the facility the periods of increased risk of infestation, or at least of more insect activity, were related with environmental conditions, particularly with ambient temperature. The temperature (ºC) and relative humidity (%) inside the rice mill and population fluctuations of the insects during trials are presented in the Figure 2.

A total of 82 data sets were collected with mean densities ranging from 0.04 to 4.68 *T. castaneum* adults per trap and 86 data sets were collected with mean densities ranging from 0.04 to 16.28 *Sitophilus* spp adults per trap. The replacement of the food-baited Dome Traps with *Sitophilus* pheromone baited PC Traps increased the trap catches of weevils by four times: average of 1.9 weevils caught per trap in the last week using Dome trap without pheromone compared with 9.6 in the first week with PC trap with pheromone. For *T.*

castaneum number captured tended to be lower after the change in trap type: 0.32 adults/trap (8 September 2006) before and 0.16 adults/trap (8-15 September 2006) after switch.

During experiments the mean temperature ranged between 13.5ºC (December 2006) and 25.0ºC (July 2006) and the relative humidity between 49.3% (August 2006) and 79.6% (November 2006). Comparing the environmental conditions in the factory and packaging sections (Table 2) the temperature and relative humidity did not differ between both localities and with the global data. Temperatures below 18ºC, when the insect activity slows (Navarro and Noyes, 2002), were recorded between December and March (Figure 2) and less than one insect on average was caught per trap during this period.

Even if the trap catches had an increase tendency with the increasing of temperature, there was not a direct relationship between temperature and insect' population fluctuations (r^2= 0.14 for *Sitophilus* and r^2= 0.13 for *T. castaneum*).

The increase of weevils' catches is related with the replacement of the food lure by a lure with its synthetic aggregation pheromone, the sitophilate, which has been demonstrated by Phillips et al. (1987). However, aggregation pheromones do not act as efficiently as most sex pheromones (Evans, 1984). Likhayo and Hodges (2000) developed experiments using flight traps with open vertical baffles and refuge traps in the form of rectangular cardboard boxes to catch *Sitophilus zeamais* and *S. oryzae* in Kenya. Traps baited with the aggregation pheromone (sitophilure) were field tested and these studies confirmed that *S. zeamais* and *S. oryzae* are attracted to sources of sitophilure when walking or flying.

3.3. Relationship between step in rice processing and insect infestation

The number of adults of Sitophilus spp. and *T. castaneum* caught in traps was analyzed following the process from paddy reception to packaging the final product (Table 1 and Figures 3 and 4). For *Sitophilus* spp., few adults were captured in the paddy reception area, but the counts increased in the mill near the first cleaning actions and peaked at roller mills. After this processing step, the number of individuals trapped tended to decrease with two smaller peaks recorded close to the rice polishers and white rice classifiers sieves. Some weevils were found in the packaging section of the rice mill that may have dispersed from other areas or originated from previous infested rice kernels.

Examining trap counts of *T. castaneum'* adults, a peak of individuals was recorded in traps close to de-stoning and plan shifting machinery which suggests that some of the external population can be retained by the cleaning machines (Figure 4). In contrast to rice weevils, the trap catches of *T. castaneum* tended to drop close to the roller rice mills, and then increase again close to classifying sieves where the broken rice is separated from the whole grain, and a another smaller peak of catches was recorded close to a rice polisher. Such as *Sitophilus* spp., *T. castaneum* was also present in the packaging section.

The cleaning machines are an important step to reduce external but not internal feeders. The internally feeding insects were also present close to roller rice mills and rice polishers, while external feeders remain mainly close to the equipments producing dust and broken rice.

These species were well established and not suppressed when managers decided treat with insecticides or applied sanitation measures. The rice facility is an old construction and structurally, we can find cracks, holes, loose joints in the interior walls floor and overheads areas not properly sealed which difficult the exclusion of insects. According Campbell et al. (2010 a,b), individuals can survive to treatments, including eggs and early instars not detectable in traps immediately, immigration, and the progeny of the survivors and immigrants; coupled with the influence of environmental conditions and management tactics.

3.4. Insects dispersion patterns assessment using Taylor's power law regression

Taylor's power law were used to identify the dispersion patterns of *Sitophilus* spp. and *T. castaneum* and to develop a sequential sampling plan for *Sitophilus* spp.

To determine Taylor's power law parameters A and b, 71 sample counts for *Sitophilus* spp and 82 sample counts for *T. castaneum* were taken. The number of traps was constant during experiments and the results are shown in Table 3.

The linear regression model fit very well almost all data for *Sitophilus* spp. (r^2=0.96) and for *T. castaneum* (r^2=0.92). Results obtained from Taylor power law regression showed similar A values for all species and for both types of trap. The b values were stable (1.20-1.76), and greater than 1 (b=1.44, $P<0.05$, t=40, df=70 for *Sitophilus* spp. and b=1.40, $P<0.05$, t=27.1, df=81 for *T. castaneum*), indicating an aggregated distribution (Southwood, 1978).

To develop the sequential sampling plan, for Sitophilus spp., the two parameters estimated from this method were useful for the calculation of the stop lines, using the Green´s stopping rules.

The overlapping 95% confidence interval for *A* and *b* showed that estimates were similar when data from all mean-variance pairs were used in the regression or the mean-variance pairs using Dome or PC Floor traps were compared.

3.5. *Sitophilus* spp. density estimation using Green's fixed precision sampling plan

Sitophilus spp. density estimation using Green's fixed precision sampling plan the parameters *A* and *b* (A=4.06 and *b*=1.44) of Taylor's power law were used to calculate the stop lines for this sequential sampling plan.

The results for *Sitophilus* spp. density estimation using Green's method are shown at Figure 4. Sequential sampling plan was developed for fixed-precision levels (*d*) of 0.20, 0.25, 0.30 and 0.35 for adults of *Sitophilus* spp.

These stop lines show that the higher the insect population the less traps are needed for estimating mean densities. At densities of 0.5 insects per trap, the number of traps needed increased with increasing precision level (i.e., lower d value): 22 traps (d=0.20), 14 traps (d=0.25) and 10 traps (d=0.30), and seven traps (d=0.35) for *Sitophilus* spp. adults.

3.6. Resampling Validation of Sampling Plan (RVSP)

The computer program RVSP was used to calculate actual error rates of the sampling plan and applied to validate the Green's stopping rule method for the key pest (Green 1966; Naranjo and Hutchison, 1997; O'Rourke and Hutchison, 2003).

The RVSP requires the use of independent data sets for validation and 15 data sets were randomly selected, to generate data analysis.

The analysis used with the precision (*d*) set at 0.20, 0.25, 0.30 and 0.35 resulted in estimated sample number (ESN) of 57, 37, 26 and 20 traps, respectively, with the range of mean density of *Sitophilus* spp. from 0.60 to 14.6 adults/trap/week (Tables 4, 5, 6 and 7).

A precision level of 0.20, increases the number of traps with pheromone, to 57 devices, increases the cost of management and created and the amount of pheromone released which might affect insects' captures. Using between 0.25 and 0.35 precision levels seemed to be most feasible levels, balancing the costs in materials and labour along with the potential for increased interference among traps at high trap densities, but lose accuracy at the lowest trap density (20 traps).

Conclusion

This facility provides an ideal environment for the development of stored-product insects due to product residues in pieces of equipment, lack of accessibility to clean certain pieces of equipment, and structural features where food residues accumulate.

Several insects species associated with stored product were collected and identified in this study but the recognized key-pest were *Sitophilus* spp. Followed by *T. castaneum*. Although there is not a direct relationship between population fluctuations and environmental conditions the risk of infestation occurs during the warm seasons. *Sitophilus* spp. can surpass all the milling process mainly because they develop inside the kernel. Due to its economical importance a consistent and cost-effective sampling methods are critical to the development of monitoring systems for pest management. The sampling plan implemented applied either Dome traps with food-lure or PC traps with pheromone-lure. All data sets fit well Taylor's power law method and the parameters *A* and *b* showed similarity between data from all mean-variance pairs used in the regression and from the mean-variance pairs using both type of traps.

Validation and evaluation of these sampling plans are fundamental for their implementation in stored products. The major benefit of a resampling approach is that the spatial distribution of the insect population is defined by actual field data rather than a theoretical model. By using independent field data it is possible to simultaneously test the accuracy of the Taylor's power law used for the sampling plan the sampling error associated with Green's method for the sequential sampling plan. The major limitation of a resampling approach is that additional field data, independent of that use to develop the sampling plan, must be collected. Ideally, these independent data also need to cover the range of population densities under which the sample plan will likely be used (Naranjo and Hutchison, 1997).

The fixed-precision sequential sampling plan for *Sitophilus* spp. inside the rice mill can be useful for rapid estimation of insects density to help the decision-making of treat when is needed and assess the efficacy of the control tactics (Bartels and Hutchison, 1995) as fumigation and preventive tactics as cleaning actions.

Based on the established fixed precision sequential sampling plan, our corrent sample size would yield a precision level of 0.30. This relative low precision migh indicate that the current sampling plan can be improved adding more traps particularly at the reception and/or at package sections. A precision level of 0.25 is generally accepted for pest sampling in IPM programs (Southwood 1978). Increasing the number of traps to 37 devices to achieve 0.25 precision level could be obtained.

Nowadays eight rice mills are working in Portugal. Studies were conducted in three rice fields, nine paddy rice warehouses and three rice mills, in order to determine the insect species associated with stored rice and their abundance, distributed through two of the three main rice production regions: Mondego, Tejo and Sado Valleys. At the rice mills the main insects caught were commodity feeders and the key-pest in Portugal is *Sitophilus zeamais* Motschulsky followed by *S. oryzae* (L.) (Coleoptera, Curculionidae), *Tribolium castaneum* (Herbst)(Coleoptera, Tenebrionidae) and Cryptolestes *ferrugineus* (Stevens) (Carvalho et al., 2004, 2011; Pires et al*.*, 2008). The results have been useful to help the decision-making. This study can contribute as step for validation and evaluation the sampling programs applied in these facilities and similars. It is highly recommended computer simulation of the performance of the sampling plan as a routine part in order to optimize sample size, save time and money and minimize the error of not treat when is necessary and treat when is not needed. Further simulations using data collected from rice facilities can provide adjustments in the stop lines. This fixed-precison sequential sampling plan for *Sitophilus* spp populations on rice revealed useful for IPM purposes.

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Table 1 – Insect species, common name, total (T) of adults caught and proportion (P) of the total in the rice facility captured in different areas: reception, factory and package

		Reception		Factory		Packaging		Total	
Order, Family, species	Common name	T	P	T	P	T	P	T	P
Coleoptera									
Curculionidae									
Sitophilus oryzae (L)	rice weevil	290	0.03	6782	0.76	1024	0.11	8096	0.90
Sitophilus zeamais Motschulsky	maize weevil								
Tenebrionidae									
Tribolium castaneum (Herbst)	red flour beetle	54	0.01	606	0.07	65	0.01	725	0.08
Gnathocerus cornutus (F.)		5	0.00	110	0.01	27	0.00	142	0.02
Laemophloeidae									
Cryptolestes ferrugineus (Stephens)	rusty grain beetle			22	0.00			22	0.00
Bostrychidae									
Rhyzopertha dominica (F.)	lesser grain borer	1	0.00	5	0.00			5	0.00
Mycetophagidae									
Typhaea stercorea (L.)	hairy fungus beetle	1	0.00					1	0.00
Cryptophagidae									
Cryptophagus spp.	fungus beetle	1	0.00					1	0.00
Carabidae		2	0.00					2	0.00
Hymenoptera									
Pteromalidae									
Anisopteromalus calandrae (Howard)		7	0.00					7	0.00
Total		361	0.04	7525	0.84	1116	0.12	8963	1.00

Table 2 – Environmental conditions of temperature and relative humidity (mean ± SE, minimum and maximum) inside the factory and package sections and overall

Temperature and Relative humidity		Factory	Packaging	All
Temperature	Mean ± SE	18.78 ±0.84	18.84±0.85	18.81±0.85
	Min.	13.44	13.46	13.45
	Max.	25.16	24.90	25.03
Relative Humidity	Mean ± SE	60.51±1.81	60.54±1.77	60.53±1.79
	Min.	49.42	49.27	49.35
	Max.	80.06	79.10	79.58

Table 3 – Taylor's Power Law estimates for adults of *Sitophilus* spp. and *Tribolium castaneum* caught by pitfall traps in the rice plant

Species	Type of traps	Number of traps[a]	n[b]	*TPL* estimate (95% confidence interval)[c] A[d]	b	R^2
Sitophilus spp.	Both traps	25	71	4.06 (3.00-5.12)	1.44 (1.40-1.47)	0.96
T. castaneum		25	82	3.37 (2.83-4.01)	1.40 (1.30-1.49)	0.92
Sitophilus spp.	Dome traps	25	35	4.72 (3.64-5.79)	1.56 (1.50-1.62)	0.95
T. castaneum		25	48	3.16 (2.67-3.76)	1.40 (1.26-1.46)	0.94
Sitophilus spp.	PC Floor	25	36	3.51 (2.23-4.79)	1.48 (1.35-1.61)	0.79
T. castaneum		25	34	4.22 (2.72-6.56)	1.51 (1.29-1.73)	0.86

[a] the trapping duration was one week

[b] number of mean-variance pairs used in the regression

[c] The 95% confidence interval by multiplying standard error of A or *b* with $z_{\alpha/2}$ at α=0.05 [1.96].

[d] Antilogarithm of *a* ($A=10^a$)

Table 4 - Resampling results for validation of Green's fixed-precision sequential sampling plan for *Sitophilus* spp. with pre-set precision (d)=0.20[a]

Data set	Observed density[b]	Average statistics for 500 sequential sampling simulations (d=0.20)						
	(N=25)	Density	Precision (d)			Sample size		
				Max	Min	ESN	Max	Min
1	0.60	0.64	0.25	0.31	0.20	142	214	82
2	2.76	2.70	0.27	0.34	0.21	64	98	40
3	1.76	1.86	0.21	0.26	0.17	77	115	53
4	3.72	3.96	0.23	0.31	0.15	51	75	34
5	1.88	2.03	0.23	0.27	0.18	74	110	47
6	4.00	4.12	0.18	0.23	0.13	49	67	37
7	5.84	5.88	0.14	0.19	0.10	40	56	30
8	3.08	3.19	0.14	0.18	0.11	56	72	44
9	1.92	1.96	0.16	0.20	0.13	74	92	58
10	2.84	2.96	0.25	0.30	0.20	60	**91**	**40**
11	7.24	7.69	0.29	0.35	0.21	**36**	60	21
12	14.56	15.00	0.17	0.22	0.12	24	32	18
13	7.96	7.84	0.17	0.21	0.13	35	45	26
14	6.96	7.03	0.14	0.18	0.10	36	48	29
15	6.28	6.45	0.17	0.23	0.12	38	52	28
Overall mean			0.20			57		

a) —— , where T_n=cumulative number of individuals sampled, A =4.06; b = 1.44; n=number of samples (Green, 1970).

b) Observed mean density values taken from field-collected independent validation sets (mean number of Sitophilus adults/trap), not used to develop the sampling plan.

Table 5 - Resampling results for validation of Green’s fixed-precision sequential sampling plan for *Sitophilus* spp. with pre-set precision (d)=0.25[a]

Data set	Observed density[b]	Average statistics for 500 sequential sampling simulations (d=0.25)						
	(N=25)	Density	Precision (d)			Sample size		
				Max	Min	(ESN)	Max	Min
1	0.60	0.66	0.31	0.39	0.21	91	153	56
2	2.76	2.77	0.33	0.42	0.24	41	71	24
3	1.76	1.87	0.26	0.35	0.20	50	85	34
4	3.72	4.06	0.29	0.40	0.17	33	53	21
5	1.88	2.06	0.28	0.35	0.21	48	88	32
6	4.00	4.26	0.22	0.30	0.14	31	47	20
7	5.84	5.97	0.18	0.25	0.12	26	34	20
8	3.08	3.24	0.18	0.23	0.13	36	49	24
9	1.92	1.96	0.20	0.26	0.15	48	65	34
10	2.84	2.97	0.31	0.38	0.24	40	64	24
11	7.24	7.90	0.35	0.46	0.19	24	42	13
12	14.56	14.94	0.21	0.29	0.11	16	23	11
13	7.96	8.20	0.21	0.27	0.14	22	32	16
14	6.96	7.02	0.18	0.22	0.11	24	30	17
15	6.28	6.57	0.21	0.30	0.13	25	36	18
Overall mean			0.25			37		

a) —— , where T_n=cumulative number of individuals sampled, a =4.06; b = 1.44; n=number of samples (Green, 1970).

b) Observed mean density values taken from field-collected independent validation sets (mean number of Sitophilus adults/trap), not used to develop the sampling plan.

Table 6- Resampling results for validation of Green’s fixed-precision sequential sampling plan for *Sitophilus* spp. with pre-set precision (d) =0.30[a]

Data set	Observed density[b] (N=25)	Average statistics for 500 sequential sampling simulations (d=0.30)						
		Density	Precision (d)			Sample size		
				Max	Min	(ESN)	Max	Min
1	0.60	0.67	0.37	0.46	0.23	64	111	34
2	2.76	2.85	0.40	0.50	0.29	29	54	15
3	1.76	1.88	0.32	0.41	0.23	35	57	22
4	3.72	3.96	0.34	0.52	0.18	24	42	14
5	1.88	2.11	0.34	0.45	0.21	34	62	18
6	4.00	4.27	0.26	0.37	0.17	22	34	15
7	5.84	6.08	0.21	0.32	0.10	18	26	13
8	3.08	3.22	0.22	0.29	0.15	26	39	19
9	1.92	1.96	0.24	0.34	0.17	34	50	22
10	2.84	3.06	0.37	0.47	0.22	28	45	15
11	7.24	8.17	0.41	0.58	0.19	17	34	10
12	14.56	14.84	0.26	0.37	0.11	11	18	10
13	7.96	8.01	0.25	0.35	0.14	16	24	11
14	6.96	7.10	0.21	0.29	0.11	17	24	12
15	6.28	6.47	0.25	0.39	0.14	18	25	11
Overall mean			0.30			26		

a) —— , where T_n=cumulative number of individuals sampled, A =4.06; b = 1.44; n=number of samples (Green, 1970).

b) Observed mean density values taken from field-collected independent validation sets (mean number of Sitophilus adults/trap), not used to develop the sampling plan.

Table 7 - Resampling results for validation of Green's fixed-precision sequential sampling plan for *Sitophilus* spp. with pre-set precision (d)=0.35[a]

Data set	Observed density[b]	Average statistics for 500 sequential sampling simulations (d=0.35)						
	(N=25)	Density	Precision (d)			Sample size		
				Max	Min	(ESN)	Max	Min
1	0.60	0.67	0.43	0.55	0.25	49	86	23
2	2.76	3.02	0.45	0.62	0.27	22	47	11
3	1.76	1.94	0.36	0.48	0.24	26	47	16
4	3.72	4.15	0.38	0.65	0.20	18	31	10
5	1.88	2.14	0.39	0.63	0.27	25	49	13
6	4.00	4.35	0.30	0.46	0.16	17	27	10
7	5.84	6.09	0.25	0.37	0.12	14	23	10
8	3.08	3.27	0.25	0.36	0.13	19	31	13
9	1.92	1.99	0.28	0.38	0.16	25	40	15
10	2.84	3.19	0.42	0.60	0.18	21	46	10
11	7.24	7.99	0.48	0.73	0.19	13	27	10
12	14.56	14.40	0.28	0.44	0.11	10	13	10
13	7.96	7.89	0.29	0.41	0.17	12	22	10
14	6.96	7.21	0.24	0.34	0.13	12	20	10
15	6.28	6.65	0.29	0.45	0.15	13	21	10
Overall mean			0.34			20		

a) —— , where T_n=cumulative number of individuals sampled, A =4.06; b = 1.44; n=number of samples (Green, 1970).

b) Observed mean density values taken from field-collected independent validation sets (mean number of *Sitophilus* adults/trap), not used to develop the sampling plan.

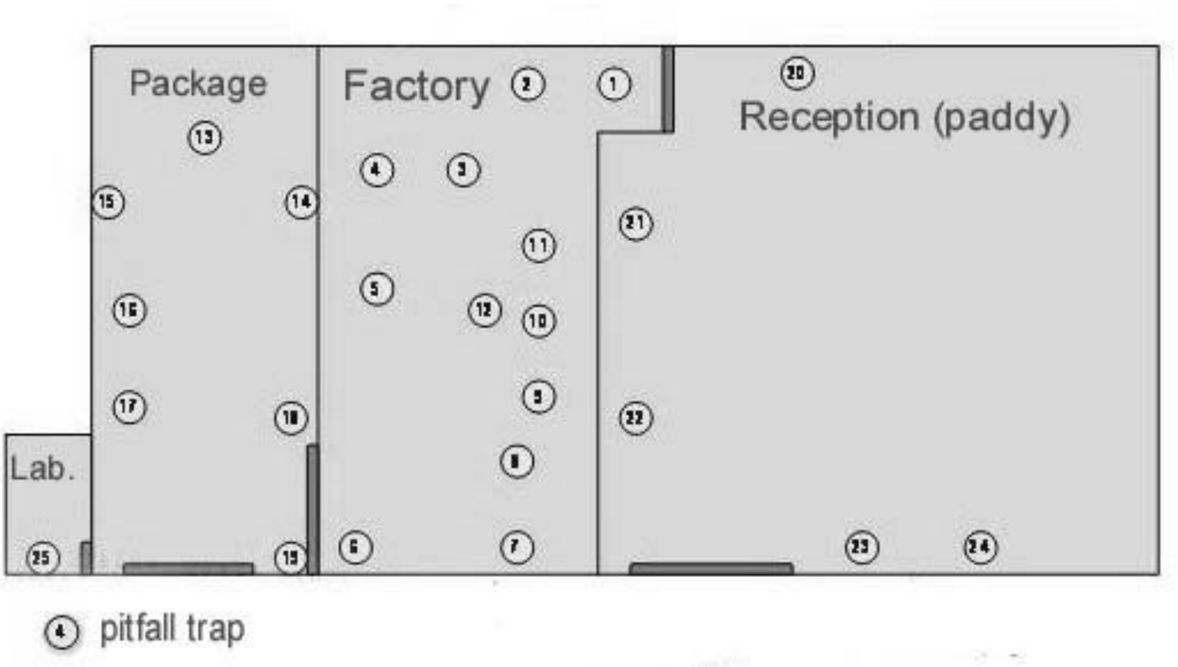
Package
Factory
Reception (paddy)
Lab.
2
1
20
13
4
3
15
14
21
11
16
5
12
10
17
9
18
22
8
25
19
6
7
23
24
4 pitfall trap

Fig. 1

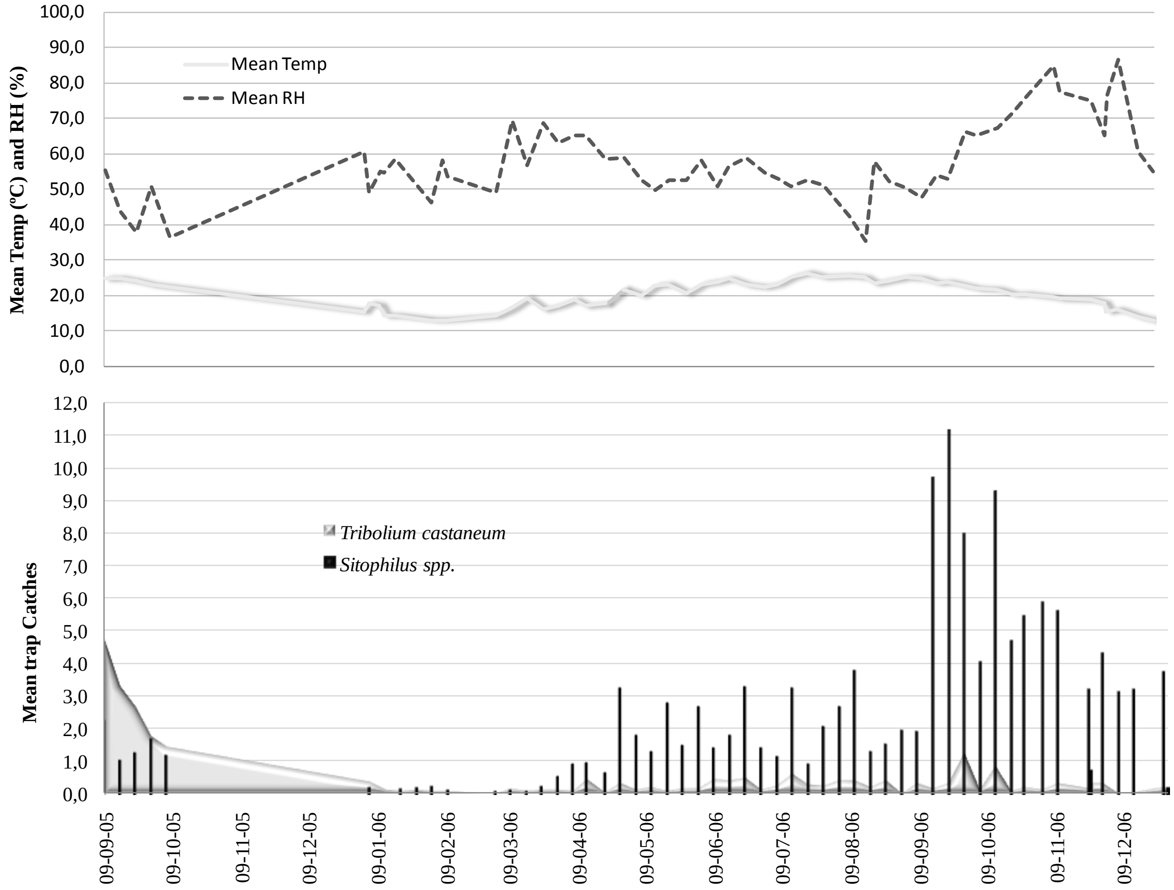
Mean Temp (ºC) and RH (%)
100,0
90,0
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
Mean Temp
Mean RH
Mean trap Catches
12,0
11,0
10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Tribolium castaneum
Sitophilus spp.
09-09-05
09-10-05
09-11-05
09-12-05
09-01-06
09-02-06
09-03-06
09-04-06
09-05-06
09-06-06
09-07-06
09-08-06
09-09-06
09-10-06
09-11-06
09-12-06

Figure 2

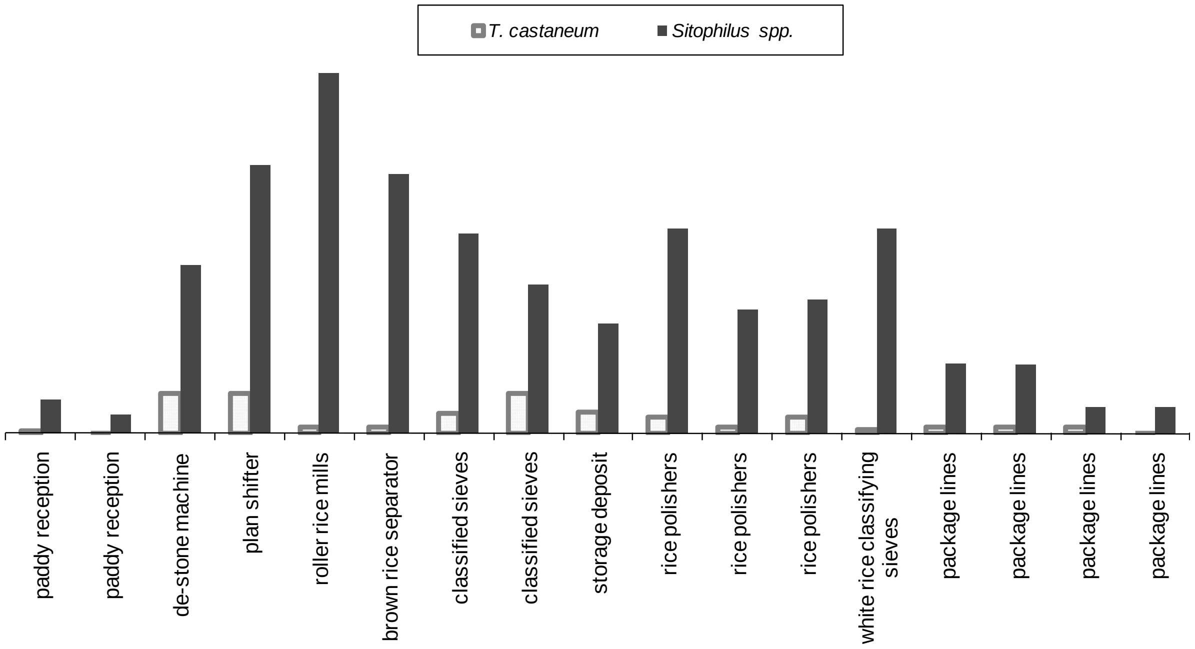
T. castaneum
Sitophilus spp.
paddy reception
paddy reception
de-stone machine
plan shifter
roller rice mills
brown rice separator
classified sieves
classified sieves
storage deposit
rice polishers
rice polishers
rice polishers
white rice classifying sieves
package lines
package lines
package lines
package lines

Figure 3

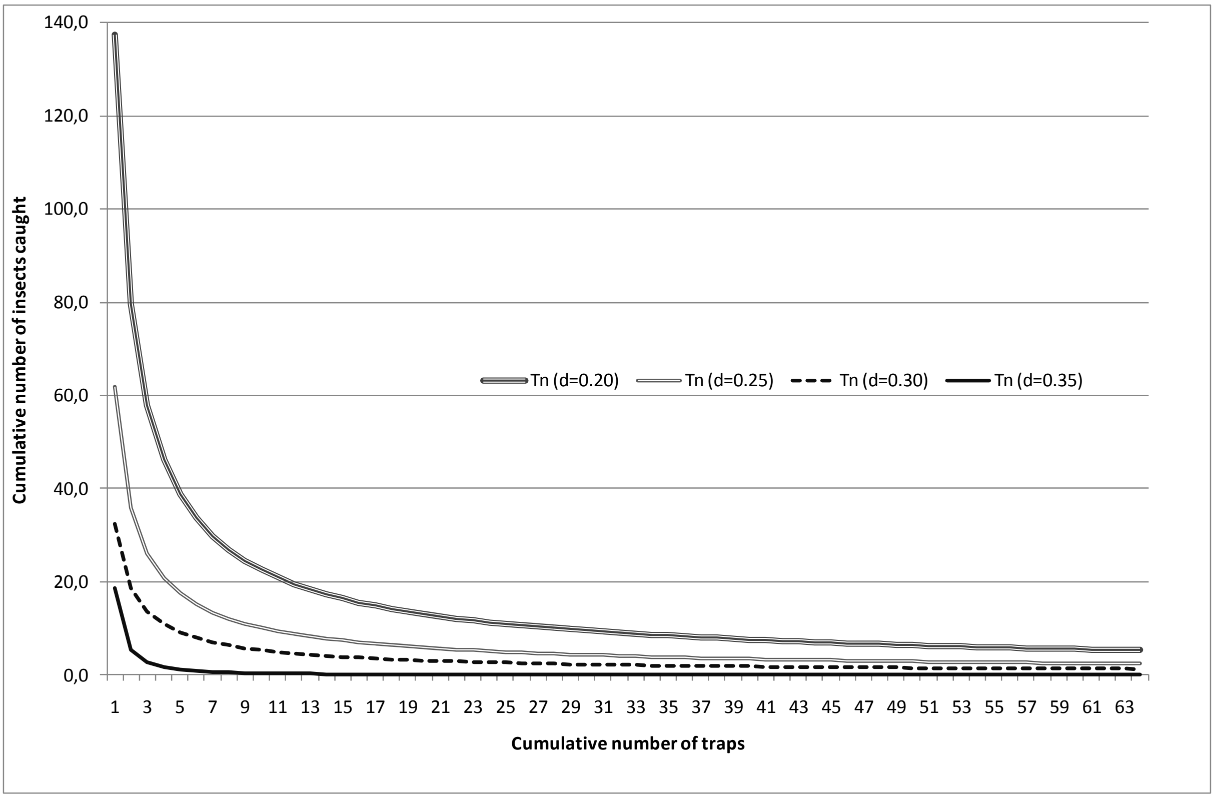
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
Cumulative number of insects caught
Tn (d=0.20)
Tn (d=0.25)
Tn (d=0.30)
Tn (d=0.35)
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63
Cumulative number of traps

Figure 4

Figure 1 - Layout of the rice mill showing trap locations. 1) De-stoning machine; 2) sifter; 3-4) sieves for classifying contaminants; 5) storage deposit; 6-19) floor close to door; 7) roller stands; 8) brown rice separator; 9-10-11) rice polishers; 12) white rice classifying sieves; 13 to 17) package lines; 18) floor; 20) big bags with broken rice; 21-22) paddy receiving area; 23-24) cyclones (aspirators); 25) rice mill laboratory to house retainer samples.

Figure 2 - Mean trap catches of *Sitophilus* spp. and *Tribolium castaneum* and the environmental conditions [mean temperature (ºC) and relative humidity (r.h.)] registered by week in the rice mill during trials.

Figure 3 - Total of adults of *Sitophilus* spp. and *Tribolium castaneum* caught along the rice process. Inside the packaging area some rice weevils can be presented which suggests that they can remain around and infest the final product.

Figure 4 - Fixed precision stops lines for sequential estimation of *Sitophilus* spp. density. The precision (D) was expressed as the ratio of the standard error of the mean to the mean