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Evaluation of sampling units and sampling plans for adults of *Cryptolestes ferrugineus* (Coleoptea: Laemophloeidae) in stored wheat under different temperatures, moisture contents, and adult densities

Fuji Jian [a,b], Ron Larson [b], Digvir S. Jayas [a,*], Noel D.G. White [c]

[a] Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB R3T 5V6, Canada
[b] OPIsystems Inc., 1216 36th Avenue, NE, Calgary, Alberta, T2E 6M8, Canada
[c] Agriculture and Agri-Food Canada, Cereal Research Centre, 195 Dafoe Road, Winnipeg, MB R3T 2M9, Canada

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A B S T R A C T

Development and evaluation of optimum size and number of sample units is required for cost-effective management of stored grain beetles. In this study, we evaluated the sampling parameters and accuracy of insect density detection and estimation, developed the optimum size and number of sample units, and conducted a feasibility study of the insect detection and density estimation. The measured insect densities in 92% of random samples were less than the introduced insect densities and 67.4 ± 10.8% of random samples did not contain adults when the introduced insect density was 0.1 A/kg (adult/kg). If the random sampling technique was used and 15% of the stored wheat bulk was sampled, 72% of determined means of insect densities of the sampling sets were lower than the introduced insect densities. Increasing the size of sample units did not improve the accuracy of the estimation of insect densities; however, it did considerably increase the probability of insect detection when insect densities were lower than 1.0 A/kg. We recommend at least 7 kg per sample unit for insect detection (especially when insect densities < 0.1 A/kg) and the optimum number of sample units with 15 kg grain per unit should be >24 for a fixed precision of 0.35 when insect densities < 0.1 A/kg. This might be a challenge for grain storage practice. Therefore, using sampling technique to estimate insect densities and detect insects at low insect densities (<0.1 A/kg) might not be practicable.

1. Introduction

Insect infestation is an important quality factor of stored grain and represents a serious and continuing problem for the grain industry. To detect insects inside a stored grain bulk, one of the commonly used methods is to sample by pushing a grain probe (mechanical or manual), trier, or spear, and immediately withdrawing it; then separating the insects from the sampled grain by sifting. This procedure usually provides less than 0.5 kg of grain (manual sampling) or 2.75 kg of grain (mechanical sampling) sample per unit, and is used by farmers, elevators, and grain buyers (Wright and Mills, 1983; Perez-Mendoza et al., 2004). The main reasons for using this sampling unit are: 1) it is the traditional method to test grain quality such as moisture content, dockage, fine material, broken kernel, and mould; so it is convenient to test all factors in one sample; 2) obtaining a larger sample size (≥2.75 kg) is time consuming and is economically and practically impossible under some situations such as sampling grain from large silos; and 3) it gives a quick indication of the number and species of insects that are present in the samples (Wilkin and Fleurat-Lessard, 1990; Hagstrum, 2000; Subramanyam and Harein, 1990). However, it poses several problems (White and Loschiavo, 1986). One of the problems is that samples are small and not statistically representative, especially considering the typically non-uniform distribution of insects (White and Loschiavo, 1986). For example, at an infestation of 100 insects/t, a total 10 kg sample per 10 t of grain will not contain insects 91% of the time; at 1760 insects/t, a sample of 0.4 kg per 12 t will not contain insects 50% of the time (Johnston, 1981). This method has been reported to be inaccurate when detecting population densities at ≤ 4 insects per kilogram of grain (Wright and Mills, 1983; White and Loschiavo, 1986; Wilkin and Fleurat-Lessard, 1990).

A reliable sampling plan is essential when developing sampling protocols for stored-grain management. Sampling plans that use a fixed number of sample units have been developed for estimating the density or determining the infestation level of stored-product insects in bulk grain (Hagstrum et al., 1985; Hodges et al., 1985;

* Corresponding author.
E-mail address: Digvir_jayas@umanitoba.ca (D.S. Jayas).

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Subramanyam and Harein, 1990; Subramanyam et al., 1997). Sampling plans can also be developed using sequential or cluster methods. Sequential or cluster sampling plans use variable numbers of sample units, and therefore are less expensive than methods based on a fixed number of sample units (Waters, 1955). Subramanyam et al. (1997) developed a sequential sampling plan for *Cryptolestes ferrugineus* in infested farm-stored wheat. They recommend using a sequence sampling plan to estimate the economic threshold density (1 insect/0.5 sample unit) of *C. ferrugineus*. Athanassiou et al. (2003) calculated the required number of sampling units for a given level of precision. The developed sampling plans mentioned above are based on the ≤0.5 kg sampling units. All of the published studies are also based on unknown insect densities. Jian et al. (2011) found the b value associated with Taylor's power law at 0.1 Adults/kg (referred to as A/kg) insect density was 1.2–1.6-fold higher than that at 1.0 or 10.0 A/kg insect densities. The b value of Taylor's power law is the parameter used to categorize uniform, random, and aggregated spatial patterns of insect distribution based on b being < 1, $= 1$, and >1, respectively. The sequential sampling plan developed by Subramanyam et al. (1997) was based on Taylor's power law. It is not known if this developed plan is accurate at low insect densities. Jian et al. (2011) also found the degrees of aggregation of adult *C. ferrugineus* using large sampling units (15 and 45 kg) were different than some previous research using small sampling units (≤2.75 kg). If the degrees of aggregation of insects are different due to different sample units, larger laboratory scale tests with different sample units, known insect densities, and high percentage of sampled grain are required. Insect numbers at high density and in the vertical direction were better correlated than that at low insect density and in the horizontal direction (Jian et al., 2011). A temporal continuous property might not exist. This spatial and temporal distribution might influence the sampling parameters.

Sampling serves as the primary basis of integrated pest management (IPM). To improve the accuracy of the grain sampling, one of the choices is to increase the size and number of sample units. However, there is no published research to evaluate either the optimum size of sample units or the effect of sample units on the detection of insects and their density estimation in stored grain.

The objectives of this study were to evaluate: 1) the sampling parameters and accuracy of insect density detection and estimation by using large sampling units (45 kg or 15 kg per unit) and introduced insect densities; 2) the optimum size and number of sample units; and 3) the feasibility of insect detection and density estimation using a sequential sampling plan at low insect density (<1 A/kg).

2. Materials and methods

2.1. Collection of the sampling data

Experimental details for data collection are given in Jian et al. (2011). The experiment was conducted in plastic bins (1.5 m diameter and 1.5 m high) filled with about 1.5 t wheat. Wheat was stored under the following constant temperature and moisture conditions: 20, 25, and 30 °C; and 11%, 13% and 15% (wet basis). Three insect densities (0.1, 1.0, and 10.0 A/kg) were tested at each grain temperature and moisture content. The grain samples were collected at five locations (center and four half radii locations) inside the bin (Fig. 1 in Jian et al., 2011). At each location, the grain in three layers (top, middle, and bottom) was removed sequentially (Fig. 1). The mass of wheat from each layer was about 15 kg. During the data analysis, this 15 kg sample was referred to as one subunit. Three subunits, which were at the same location but at different layers vertically, were pooled and referred to as one primary unit.

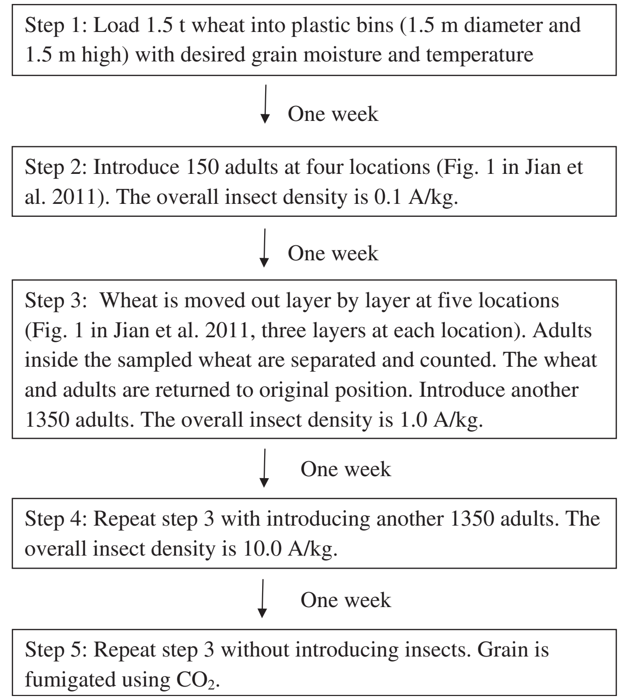

Fig. 1. Diagram of the sampling procedure conducted by Jian et al. (2011). A/kg refers to adults per kilogram of wheat.

The mass of each primary unit was about 45 kg. Therefore, one sampling set was composed of five primary units or 15 subunits. A total of 36 sampling sets were collected in the entire study. There were totally 540 subunits. There were 270 subunits at 20 °C and 135 subunits at 25 or 30 °C. There were 180 subunits at each introduced insect density. The performance between the subunits and primary units were compared.

2.2. Accuracy of insect density estimation and detection

To evaluate if a large sample unit can improve the accuracy of insect density estimation and detection, the following statistics were conducted (SAS, 2008): mean of insect densities and associated standard error in each sampling set, comparison between the determined and introduced insect densities (Student *t*-test), the percentage of samples with the determined insect density < introduced insect density or = 0 (non-detection rate). Percentages of under-, correct- and over-estimation of the introduced insect densities at different sample layers were calculated. The percentage of under-estimation at different layers was compared (Tukey test).

2.3. Precision of the sampling

To check if increasing the size of sampling units increases sampling precision; the sampling precision (percent relative variance, RV) was calculated as (Legg and Moon, 1994):

$$RV = \frac{100\sqrt{\frac{S^2}{n}}}{\overline{X}} \quad (2)$$

where, $\overline{X}$ is the determined mean of the sampling set (A/kg), and s^2 is the variance of the sampling set. The variance associated with the subunits in each sample set is a slightly biased estimate of population variance (Hamilton and Hepworth, 2004), because the