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Development and Evaluation of Sequential Sampling Plans for *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) Infesting Farm-stored Wheat

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Abstract—The development and evaluation of appropriate sampling plans are needed for cost-effective management of stored-product insects. Sequential sampling plans, which are based on a variable sample size, are generally more cost effective than plans based on a fixed sample size. For adults of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), we developed sequential sampling plans based on complete counts and the presence/absence of insects in 0.5 kg grain samples removed with a trier from the top 1 m of wheat stored in farm bins. Insect count data were used to develop a sampling plan for estimating the density of *C. ferrugineus* with a fixed level of precision. The presence/absence data were used to develop a sampling plan for classifying the *C. ferrugineus* infestation level relative to an action threshold. The performance of these sampling plans was evaluated (validated) using independent data sets and an IBM-PC software program specifically designed to test the plans. This is the first paper illustrating the development and evaluation of sequential sampling plans for a stored-product insect. © 1997 Elsevier Science Ltd. All rights reserved.

Key words—Binomial sampling, computer simulations, density estimation, pest control decisions, rusty grain beetle

INTRODUCTION

Sampling is an integral component of integrated pest management (IPM) (Ruesink and Kogan, 1982). Cost-effective IPM decisions require methods to estimate accurately the insect density and to determine whether the density or infestation level (proportion of infested sample units†) has exceeded a threshold to take control action. Cost-effective sampling plans for estimating the insect density and classifying the infestation level relative to a threshold can be developed using sequential methods. Sequential sampling plans use variable numbers of sample units, and therefore are less

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†A sample unit is the fraction of habitable space from which insect counts are made (see Subramanyam and Hagstrum, 1995).

expensive than methods based on a fixed number of sample units (Waters, 1955). Sequential sampling plans, in general, require about 40–60% fewer sample units than plans based on a fixed number of sample units (Sterling, 1975).

For stored-product insects, sampling plans that utilize a fixed number of sample units have been developed for estimating the density or determining the infestation level of insects in bulk grain or probe traps (Hagstrum *et al.*, 1985; Hodges *et al.*, 1985; Subramanyam and Harein, 1990; Subramanyam *et al.*, 1993). Sequential sampling plans have not been developed for stored-product insects (Subramanyam and Hagstrum, 1995). Sequential sampling plans for estimating the insect density (Hutchison, 1994) or classifying the infestation level relative to a threshold (Binns, 1994) can be developed based on the complete counts of insects in sample units (enumerative sampling) or on the presence/absence of insects in sample units (binomial sampling). The development of enumerative and binomial sampling plans for pest insects can reduce sampling costs, prevent unnecessary pest management actions and permit the evaluation of pest management tactics.

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae), is a common and abundant species infesting farm-stored grain in the U.S.A. and Canada (Sinha and Watters, 1985). For *C. ferrugineus* adults infesting farm-stored wheat, we have developed an enumerative sequential sampling plan to estimate the density and a binomial sequential sampling plan to classify the infestation level relative to an action threshold (AT). The performance of these plans was evaluated using a resampling approach (Naranjo and Hutchison, 1997). The resampling approach utilizes IBM-PC software (resampling for validation of sample plans or RVSP) specifically designed to evaluate sequential sampling plans. This software is available on request from S. E. Naranjo or W. D. Hutchison, or can be downloaded from the following World-Wide Web sites: http://gears.tucson.ars.ag.gov/wcrl/ and http://www.mes.umn.edu/ ~ vegipm/.

MATERIALS AND METHODS

Collection of C. ferrugineus *sampling data*

Farm-stored wheat in Kansas was sampled during 1983 and 1984 to collect data on *C. ferrugineus* (Hagstrum *et al.*, 1985; Hagstrum, 1987). Hagstrum *et al.* (1985) sampled wheat from four bins (5.8 or 6.4 m diameter) of 82 or 122 t capacity between 22 October 1983 and 6 January 1984. Wheat was sampled with a 1.27 m grain trier in the top 1 m of the bins. Each grain trier sample removed 0.5 kg of wheat (sample unit). The top 1 m was adequate because nearly 68% of the total *C. ferrugineus* adults sampled with a trier were found in this region of the grain mass (Hagstrum, 1989). On each of three separate occasions, 18 locations in three bin strata were sampled twice (total: 36 sample units/sampling occasion/bin). Therefore, from Hagstrum *et al.* (1985), a total of 12 data sets was available. Between July and December 1984, Hagstrum (1987) sampled two bins (4.3 m diameter bin with 27 t capacity, and 6.4 m diameter bin with 82 t capacity) holding newly harvested wheat. For each bin and sampling occasion, 22 sites in the top 1 m of the grain mass were probed with a 1.27 m grain trier. In each of the 27 and 82 t bins, sampling was performed on 11 different occasions. Therefore, from the two bins, a total of 22 data sets was available.

The presence and number of live *C. ferrugineus* adults in each sample unit (0.5 kg sample of wheat) were determined. Sample units with one or more adults were scored as 1, and units without an adult were scored as 0. From this information, the proportion of sample units with insects (P(I)) was calculated. For each bin and sampling occasion, counts of insects in all 22 or 36 sample units were used to calculate the sample mean (m) and sample variance (s^2). For the 22 data sets, m ranged from 0.091 to 7.909 insects/sample unit, s^2 from 0.185 to 121.661 and P(I) from 0.045 to 0.909. For the 12 data sets, m, s^2 and P(I) ranged from 0.028 to 8.361 insects/sample unit, 0.028 to 338.644 and 0.028 to 0.944, respectively.

During bin sampling (Hagstrum, 1987), the wheat moisture ranged from 10 to 13% and the grain temperature decreased from 32 to 1°C. The grain samples were collected from harvest until the onset of cooler temperatures, and therefore the insect densities reported here are representative of those occurring in bins under a range of environmental conditions.

Sampling plan for estimating the density of C. ferrugineus *with a fixed level of precision*

A fixed precision sequential sampling plan was developed using the 22 data sets (Hagstrum, 1987). The performance of the plan was evaluated using the 12 data sets (Hagstrum *et al.*, 1985). A fixed precision sequential sampling plan, proposed by Green (1970), was used for estimating the density of *C. ferrugineus*. In this plan, a decision to take additional sample units is terminated when a defined level of precision is achieved. This fixed precision stop line is calculated as

$$\ln(T_n) = \{[\ln(D^2/A)]/(b-2)\} + [(b-1)/(b-2)]\ln(n) \quad (1)$$

where T_n is the cumulative number of insects in the sample units and D is the precision expressed as the ratio of the standard error of the mean (SEM) to m; A and b were estimated by linear regression of $\ln(s^2)$ against $\ln(m)$ (Taylor, 1961; Southwood, 1978) based on the 22 data sets. A was calculated as e^a, where $e = 2.71828$ and a is the regression y intercept; b is the regression slope. Equation (1) was solved for different n at $D = 0.25$ and $D = 0.35$. A reasonable D is usually between 0.20 and 0.35 (Southwood, 1978; Hutchison *et al.*, 1988). A plot of $\ln(T_n)$ against $\ln(n)$ is linear (Green, 1970), whereas a plot of T_n against n is non-linear.

To use the sampling plan of Green (1970), independent sample units are taken sequentially and the numbers of insects in them are counted. The cumulative numbers of insects in the sample units (T_n) are plotted against the cumulative number of sample units (n) with reference to the stop line. Sampling stops when the stop line is crossed. T_n/n at this point estimates m. The number of insects in n sample units is used to calculate SEM. D is calculated as SEM/m.

The performance of the sampling plan of Green (1970) was evaluated by resampling the 12 independent data sets (Hagstrum *et al.*, 1985) using RVSP. Individual observations in the data files were sampled multiple times (resampling with replacement, see Naranjo and Hutchison, 1997), and the minimum sample size (n) was set at five. The performance of the plan was evaluated by examining the actual n and D values obtained from sequentially estimating *C. ferrugineus* densities at the two fixed precision levels. The actual n and D values obtained were averages of 500 resampling iterations.

Sampling plan for classifying the C. ferrugineus *infestation level relative to an AT*

The presence/absence or binomial sampling is a viable alternative when counting insects in sample units is cumbersome or expensive (Binns and Nyrop, 1992). We developed a binomial sampling plan based on the sequential probability ratio test (SPRT) (Wald, 1947). To develop a binomial SPRT sampling plan, the relationship between the proportion of sample units with one or more insects (P(I)) and the mean density (m) should be established (Jones, 1994). Establishing this relationship is important for the determination of P(I) at an economic threshold or AT.

The relationship between P(I) and m was established based on the 12 data sets (Hagstrum *et al.*, 1985). The relationship between P(I) and m was described by a non-linear regression, generated by TableCurve 2D software (Anonymous, 1994). From this relationship, P(I) at a density of 1 insect/sample unit (0.5 kg of wheat) was determined. The United States Grain Inspection, Packers and Stockyard Administration (formerly the Federal Grain Inspection Service) classifies grain as "infested" when a representative sample (1 kg) contains a minimum of 2 live adult insects (Hagstrum and Flinn, 1992). This tolerance for live insects may be used during marketing of the grain within the U.S.A. Grain buyers may monetarily penalize sellers for delivering infested grain (Barak and Harein, 1981; Reed and Worman, 1993; Kenkel *et al.*, 1994). Therefore, the federal tolerance level of 2 live adults/kg of grain represents the economic threshold (Hagstrum and Flinn, 1995). Because our sample unit was based on 0.5 kg of wheat, we used 1 insect/sample unit as the economic threshold. At the economic threshold, P(I) was 0.485 or 48.5%. However, we chose a P(I) value of 0.43 or 43% as the AT for two reasons:

1. to respond early to *C. ferrugineus* infestation and prevent it from reaching or exceeding the economic threshold;
2. to use a conservative estimate for the AT as both P(I) and m are subject to estimation errors.

Wald's binomial SPRT sampling plan was developed from information on four parameters (Binns, 1994): p_1, the upper threshold; p_0, the lower threshold, which is usually a certain fraction of p_1; α, the probability of accepting the hypothesis that infestation is $\geq p_1$ when in fact it is $\leq p_0$;

β, the probability of accepting the hypothesis that infestation is $\leq p_0$ when in fact it is $\geq p_1$ (Jones, 1994). We set p_0 5% below the AT and p_1 5% above the AT. Thus, $p_1 = 0.48$ (economic threshold) and $p_0 = 0.38$. We set $\alpha = \beta = 0.2$. The error rates need not be equal (Waters, 1955), and the values used should be based on the acceptable risk of incorrect classification. For example, if $\alpha = \beta = 0.2$, the probability of correctly classifying P(I) as being at or below the lower threshold is at least 0.8, and the probability of classifying P(I) as being at or below the lower threshold when it is at or above the upper threshold is, at most, 0.2. Based on the above thresholds and error rates, the upper (T_U) and lower (T_L) stop lines were generated using equations (2) – (5).

$$T_U = (\text{intercept}) \ln[(1-\beta)/\alpha] + n(\text{slope}) \tag{2}$$

$$T_L = (\text{intercept}) \ln[\beta/(1-\alpha)] + n(\text{slope}) \tag{3}$$

The intercept was calculated as

$$1/\{\ln[(p_1 q_0)/(p_0 q_1)]\} \tag{4}$$

where $p_0 = 0.38$ and $p_1 = 0.48$; $q_0 = 1 - p_0$ and $q_1 = 1 - p_1$. The slope was calculated as

$$\ln(q_0/q_1)/\{\ln[(p_1 q_0)/(p_0 q_1)]\} \tag{5}$$

The upper and lower stop lines were generated by plotting T_U and T_L against n. The stop lines are further apart if p_0 and p_1 are closer together and/or if α and β are set lower (Waters, 1955; Binns, 1994). Data from independent sample units are compared with these stop lines to arrive at a decision to terminate or continue sampling (see Subramanyam and Hagstrum, 1995). Sampling is terminated if a plot of the cumulative number of sample units with insects against the cumulative number of sample units crosses either the upper or lower stop line. If the upper stop line is crossed, infestation is $>$AT; if the lower stop line is crossed, infestation is $<$AT. If a plot of the data falls within the stop lines (uncertain zone), sampling continues until the upper or lower stop line is crossed.

The binomial SPRT is evaluated by examining the operating characteristic (OC) and average sample number (ASN) functions at each infestation level. Fowler and Lynch (1987) gave procedures for calculating the OC and ASN functions. The OC function indicates the probability of accepting the two hypotheses, and the ASN indicates the average number of sample units necessary to reach a decision at each infestation level (Southwood, 1978). Over a range of infestation levels, these OC and ASN functions are represented as curves. The OC function is near unity when $P(\mathrm{I}) < p_0$; it is near zero when $P(\mathrm{I}) > p_1$. The OC function is 0.5 when $P(\mathrm{I}) = \mathrm{AT}$ (for $\alpha = \beta$).

The performance of the binomial SPRT sampling plan was evaluated by examining the actual OC and ASN functions for a range of *C. ferrugineus* infestation levels based on the 22 independent data sets (Hagstrum, 1987). The RVSP software resampled each of the 22 data sets 500 times. Resampling was performed with replacement, and the minimum sample size was set at five. For each infestation level (P(I)), RVSP generated an OC and ASN value. Each ASN value was the average of 500 iterations, whereas each OC value was the proportion of 500 iterations where $P(\mathrm{I}) \leq p_0$ (Naranjo and Hutchison, 1997). The OC or ASN values were plotted against the corresponding P(I) (Plant and Wilson, 1985), and a smooth curve was fitted to the data points using TableCurve 2D software (Anonymous, 1994).

RESULTS AND DISCUSSION

Fixed precision sequential sampling plan for estimating the density of C. ferrugineus

The linear regression of $\ln(s^2)$ against $\ln(m)$ indicated that the variance increased significantly faster than the mean ($b > 1$, $P < 0.01$, two-tailed t-test; $R^2 = 0.939$). The y intercept ($a \pm$ SE) was 1.117 ± 0.105 and the slope ($b \pm$ SE) was 1.461 ± 0.084. The antilogarithm of the y intercept, A, was 3.056. A is a scaling factor related to the size of the sample unit (Southwood, 1978; Sawyer, 1989), and b is an index of dispersion, with values of $b < 1$, $b = 1$ and $b > 1$ indicating a uniform, random and aggregated sampling distribution, respectively (Taylor, 1961; Southwood, 1978). The values of A and b for *C. ferrugineus* reported here are well within the range of those reported for several stored-product insects sampled with various devices in different storage structures and commodities (see Subramanyam and Hagstrum, 1995). The estimates of A and b are important

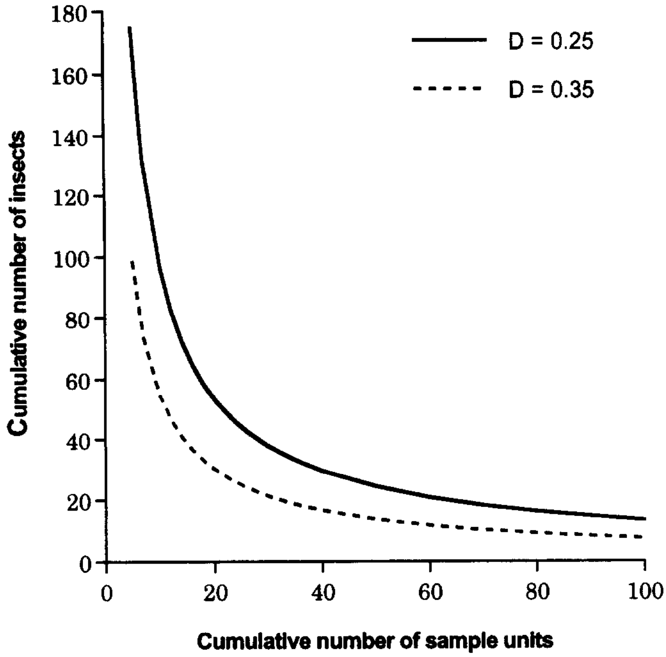

Fig. 1. Fixed precision stop lines for sequential estimation of *C. ferrugineus* density. The precision (D) was expressed as the ratio of the standard error of the mean to the mean.

for predicting the sample variance ($s^2 = Am^b$; Taylor, 1961) for independent sample means. An accurate prediction of the sample variance is essential for developing practical insect sampling plans (Nyrop and Binns, 1991; Subramanyam and Hagstrum, 1995).

The stop lines for estimating the density of *C. ferrugineus* at the two fixed precision levels (Fig. 1) indicated that the number of sample units required to cross the stop lines decreased with an increase in the insect density. About twice as many sample units were required to estimate the density at $D = 0.25$ than at $D = 0.35$.

The performance evaluated using the RVSP software indicated that to estimate 0.03 to 8.36 insects/sample unit at $D = 0.35$, 181 to 10 sample units were required (Table 1). Nearly twice as many sample units were required to estimate the insect density at $D = 0.25$ than at $D = 0.35$. The observed mean precision levels at the two fixed precision levels were variable (Table 1). The observed mean precision levels were worse than expected when estimating the following *C. ferrugineus* densities: 0.028, 2.972, 3.000, 6.222, 6.389 and 8.361 insects/sample unit. The observed

Table 1. Performance of the fixed precision sequential sampling plan for estimating the density of *C. ferrugineus*

				Fixed precision, $D = 0.25$		Fixed precision, $D = 0.35$	
Data set	m[a]	s^2[b]	Predicted s^2[c]	n[d]	Observed D[e]	n[d]	Observed D[e]
1	0.028	0.028	0.016	341	0.32	181	0.44
2	0.111	0.102	0.123	158	0.22	85	0.31
3	0.194	0.218	0.278	118	0.21	61	0.30
4	0.389	0.473	0.769	81	0.19	42	0.26
5	1.083	1.850	3.434	49	0.18	25	0.24
6	1.222	2.121	4.096	45	0.18	24	0.24
7	2.972	44.485	15.006	30	0.40	16	0.53
8	3.000	71.315	15.213	29	0.47	16	0.57
9	4.056	17.540	23.637	24	0.20	13	0.27
10	6.222	161.721	44.166	21	0.42	11	0.50
11	6.389	338.644	45.908	21	0.58	12	0.64
12	8.361	302.409	68.010	18	0.47	10	0.52

[a]Sample mean, expressed as the mean number of live *C. ferrugineus* adults/sample unit (0.5 kg of wheat). Each mean is based on 36 sample units.
[b]Sample variance.
[c]The predicted sample variance was calculated as Am^b, where $A = 3.056$, $b = 1.461$ and m is the sample mean.
[d]Mean number of sample units required to estimate m as determined by the RVSP software.
[e]Observed mean precision level for estimating m as determined by the RVSP software.

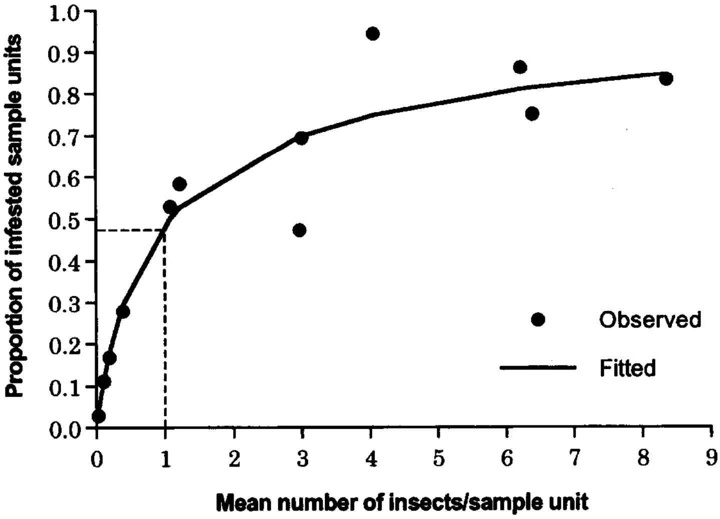

Fig. 2. Non-linear relationship between the proportion of sample units with one or more *C. ferrugineus* adults and the mean density. At the United States Grain Inspection, Packers and Stockyard Administration standard for "infested grain" of 2 insects/kg of grain or 1 insect/0.5 kg of grain (sample unit), about 0.48 or 48% of the sample units were infested.

mean precision levels were better than expected when estimating densities between 0.111 and 1.222 insects/sample unit. The observed mean precision levels were worse than expected only when the predicted sample variance at a given insect density underestimated the actual sample variance (see Table 1).

A density of 1.083 insects/sample unit (close to the economic threshold of 1 insect/sample unit) can be estimated with 49 sample units at $D = 0.25$ and with 25 sample units at $D = 0.35$ (Table 1). We recommend a fixed precision of 0.35 for estimating the economic threshold density of *C. ferrugineus*, because the observed mean precision level was 0.24 with 25 sample units. For estimating densities of less than 1.083 insects/sample unit, more than 36 sample units need to be examined. Our results suggest that the sequential sampling plan of Green (1970) is useful for estimating the density of *C. ferrugineus* at or near the economic threshold.

Binomial sampling plan for classifying the C. ferrugineus *infestation level relative to an AT*

Figure 2 shows the non-linear relationship between $P(\mathrm{I})$ and m. The regression model ($y = 1.093\{1 - 1/[1 + 2(1.093)^2(0.935x)]^{0.5}\}$) fitted the data well ($R^2 = 0.907$). For both the 22 and 12 data sets, $P(\mathrm{I})$ at a density of 1 insect/sample unit was 0.485 or 48.5%. Therefore, the relationship between $P(\mathrm{I})$ and m was established using the 12 data sets, and the performance of the binomial sequential sampling plan was evaluated using the 22 data sets.

The lower and upper stop lines for classifying the *C. ferrugineus* infestation level relative to an AT are shown in Fig. 3. The lower stop line intersects the x axis at 12 sample units. Therefore, a minimum of 12 sample units should be examined for classifying the *C. ferrugineus* infestation level. The accuracy of classifying the *C. ferrugineus* infestation levels relative to an AT was tested by RVSP using the 22 independent data sets. The OC function was near unity when $P(\mathrm{I}) \leq 0.3$ (Fig. 4). At a $P(\mathrm{I})$ value of 0.38 (p_0), the OC function was 0.851. At a $P(\mathrm{I})$ value of 0.48 (p_1), the OC function was 0.189. At $P(\mathrm{I}) > 0.48$, the OC function decreased from 0.189 to zero. At a $P(\mathrm{I})$ value of 0.43 (AT), the OC function was 0.55. Although the nominal errors were set at 0.2, the actual errors generated by RVSP may be different from 0.2. The actual α and β errors were determined from the OC curve (Fig. 4). The 1 − OC value at p_0 gives the actual α; the OC value at p_1 gives the actual β. The actual α and β values were 0.149 and 0.189 respectively. Therefore, the risk of incorrect classification with respect to the AT was less than the preset value of $\alpha = \beta = 0.2$. The OC curve indicated that the binomial sampling plan performed well in classifying the infestation levels of *C. ferrugineus* with respect to the AT. The ASN curve (Fig. 5) indicated that, to classify $P(\mathrm{I})$ between 0.045 and 0.909, an average of 7 to 51 sample units needed to be examined. The uncertainty in classifying the infestation level increases near the threshold. Therefore, the ASN is greatest near the AT. More sample units needed to be examined to cross

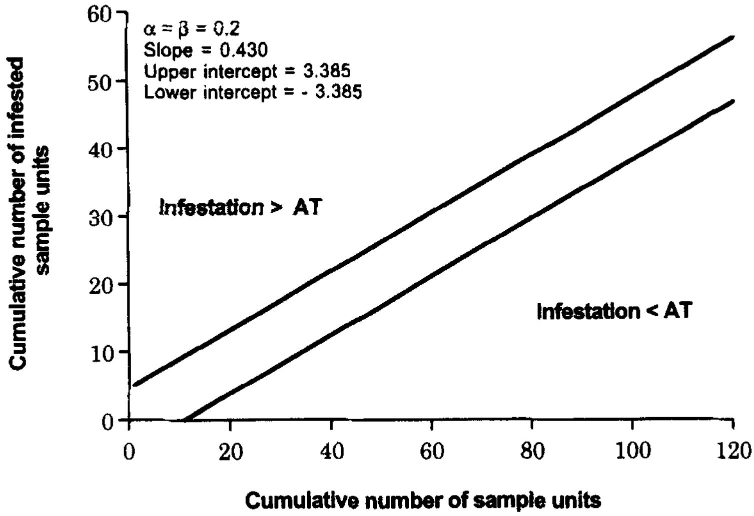

Fig. 3. Binomial sequential probability ratio test stop lines for classifying the infestation level of *C. ferrugineus* relative to the action threshold (AT).

either the upper or lower stop line when $P(\mathrm{I})$ was between p_0 and p_1 than when $P(\mathrm{I})$ was less than p_0 or greater than p_1. Each of the 22 independent data sets used to evaluate the performance of the binomial sampling plan was based on 22 sample units. Therefore, with 22 sample units, infestation levels of ≤ 0.26 or ≥ 0.6 can be correctly classified on average with respect to the AT. However, for infestation levels between 0.26 and 0.6, we can expect that more than 22 sample units will be required (Fig. 5). If more than 22 sample units are required, they should be collected after a few days or a week. Because the processing of sample units within a bin may be cumbersome, especially during a hot day or if there is insufficient bin headspace, we recommend taking at least 22 sample units from each bin on every sampling occasion following the sampling schemes outlined in Hagstrum *et al.* (1985) or Hagstrum (1987). In the laboratory, the sample units, selected at random, should be processed sequentially to classify the *C. ferrugineus* infestation level with respect to the AT.

This is the first time that sequential sampling plans have been developed and evaluated for a stored-product insect. The enumerative sampling plan is useful for determining whether the mean

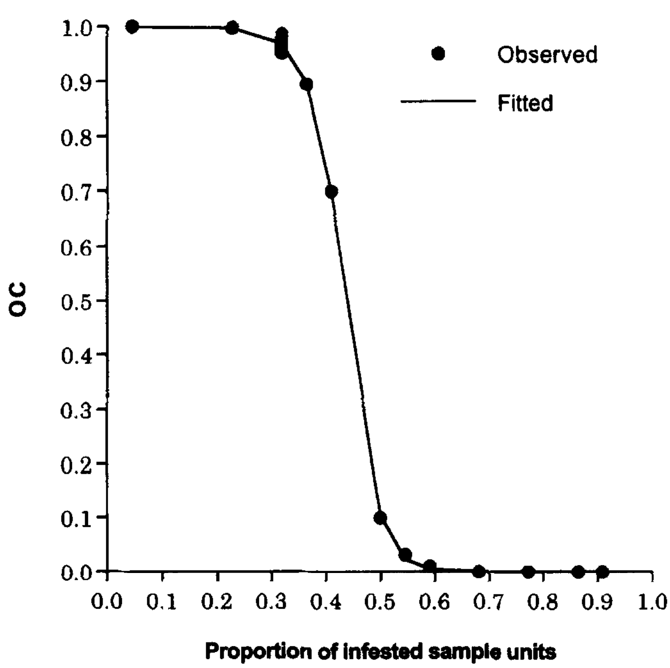

Fig. 4. Operating characteristic (OC) curve for the binomial sequential probability ratio test sampling plan ($\alpha = \beta = 0.2$, $p_0 = 0.38$, $p_1 = 0.48$).

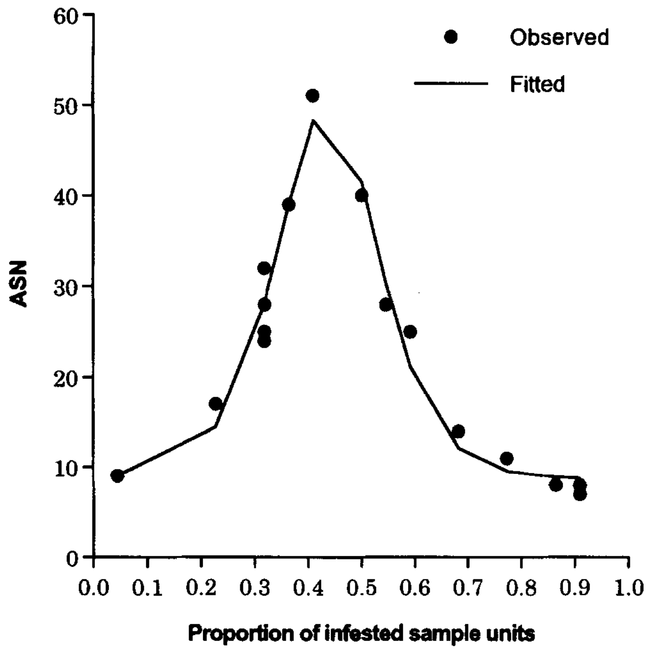

Fig. 5. Average sample number (ASN) curve for the binomial sequential probability ratio test sampling plan ($\alpha = \beta = 0.2$, $p_0 = 0.38$, $p_1 = 0.48$).

density of *C. ferrugineus* infesting the top 1 m of wheat stored in a bin is below or above the economic threshold. In situations in which the counting of adults in sample units is cumbersome, it may be appropriate to use the binomial sequential sampling plan to classify the *C. ferrugineus* infestation level relative to an AT. In the binomial sampling plan, the upper stop line represents the economic threshold. Therefore, either of the sampling plans can be used to determine whether the *C. ferrugineus* mean density or infestation level has exceeded the economic threshold. To prevent *C. ferrugineus* populations from reaching the economic injury level (Hagstrum and Flinn, 1995), a pest control measure should be applied when the mean density or infestation level is at or above the economic threshold. The use of these sequential sampling plans in IPM programs for stored-product insects will result in the application of pest control measures only when needed. We encourage researchers to develop and evaluate similar sampling plans for other economically important insects infesting stored products, and farmers/pest managers to use these plans.

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