

## **SAMPLING TO DETERMINE TREATMENT EFFICACY: DEVELOPMENT AND VALIDATION OF SEQUENTIAL SAMPLING PLANS FOR ADULTS OF THE RUSTY GRAIN BEETLE ASSOCIATED WITH FARM-STORED WHEAT**

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### **ABSTRACT**

Sequential sampling plans, based on a variable sample size, are more cost-effective than are plans based on a fixed sample size. Sequential sampling plans can be used for estimating insect density with a fixed level of precision as well as for classifying infestation level relative to an action or economic threshold. For adults of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), we developed sequential sampling plans based on complete counts and presence/absence of insects in 0.5-kg grain samples removed with a 1.27-m grain trier from the top 1 m of wheat stored in farm bins. Insect count data were used to develop an enumerative sampling plan for estimating *C. ferrugineus* density with a fixed level of precision. Presence/absence data were used to develop a binomial sampling plan for classifying the *C. ferrugineus* infestation level relative to an action threshold. The performance of these sampling plans was validated using independent data sets, and an IBM-PC software program was specifically designed to test the plans. These are the first sequential sampling plans reported for a stored-product insect.

### **INTRODUCTION**

Sampling<sup>1</sup> is an integral component of integrated pest management (IPM) (Ruesink and Kogan, 1982). Cost-effective IPM decisions require methods to both accurately estimate insect density and determine if the density or infestation level has exceeded a threshold beyond which control action is necessary. Cost-effective sampling plans for both

<sup>1</sup>For definitions, concepts and statistics pertinent to stored-product insect sampling, see Subramanyam and Hagstrum (1995).

estimating insect density and classifying the infestation level relative to an action or economic threshold can be developed using sequential methods. Sequential methods utilize a flexible sample size and are therefore generally less expensive than are methods based on a fixed sample size (Waters, 1955). In general, sequential sampling plans require about 40–60% fewer sample units than do fixed-sample size plans to attain the same precision or accuracy of classification (Sterling, 1975).

Sequential sampling plans have not been previously developed for stored-product insects (Subramanyam and Hagstrum, 1995). Sequential sampling plans for estimating insect density (Hutchison, 1994) or for classifying infestation level relative to a threshold (Binns, 1994) can both be developed based on either complete counts of insects in sample units (enumerative sampling) or the presence/absence of insects in sample units (binomial sampling). Development of enumerative and binomial sampling plans for pest insects can reduce both sampling effort and sample processing costs as well as preventing unnecessary pest management actions and permitting the evaluation of pest management tactics.

Once the sampling plans are developed, they should be validated using independent data sets or through computer simulation (Binns and Nyrop, 1992). The final step is the implementation of the sampling plans under field conditions by the end users — farmers, pest managers and scouts. Therefore, sampling plans should be simplified for these end users. Furthermore, the plans should be flexible and should also perform well in the field despite errors introduced during implementation.

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) is a common and abundant species infesting farm-stored grain in the United States and Canada (Sinha and Watters, 1985). For *C. ferrugineus* adults infesting farm-stored wheat, we developed both an enumerative sequential sampling plan to estimate density and a binomial sequential sampling plan to classify infestation level relative to an action threshold. These sampling plans were validated using a resampling approach (Naranjo and Hutchison, 1996). The resampling approach utilizes an IBM-PC computer program (Resampling for Validation of Sample Plans or RVSP) specifically designed to validate sequential sampling plans.

#### FEATURES OF THE RVSP SOFTWARE

The RVSP software evaluates two enumerative sampling plans (Kuno, 1969; Green, 1970) for estimating insect density with a fixed level of precision and two binomial sampling plans (Wald, 1947; Naranjo *et al.*, 1996) for classifying infestation level relative to a threshold. The software runs on an IBM-PC computer in the DOS environment. To use RVSP, insect counts in each sample unit from a single sampling occasion are entered in a vertical column in a single file. Data from different sampling occasions are entered as separate files (with different file names). All file names are stored in a batch file for program execution. The RVSP program simulates actual field sampling by resampling observations from these files. Therefore, resampling analyses are based on actual distributions of the insect population. A random number generator selects observations from each

field data file, and this resampling is repeated 500 times (iterations). Individual observations in a data file can be sampled once (resampling without replacement) or multiple times (resampling with replacement). We chose the latter because the program failed to resample certain files when we chose the former option. We set the minimum sample size as 5. For enumerative and binomial sampling, RVSP requires two data sets, one to fit a statistical model of spatial dispersion to data and the other to evaluate model performance (see Naranjo and Hutchison, 1996). The output containing information from all 500 iterations can be saved and used with other software applications. Summary statistics based on the 500 iterations are also printed and stored by RVSP. This public-domain software is available upon request from S.E. Naranjo or W.D. Hutchison. It can also be downloaded via the internet from the following World-Wide-Web sites: <http://gears.tucson.ars.ag.gov/wcrl/>; <http://www.mes.umn.edu/~vegipm/>.

#### COLLECTION OF *C. FERRUGINEUS* SAMPLING DATA

Farm-stored wheat in Kansas was sampled during 1983 and 1984 to collect data on *C. ferrugineus* and other insect species (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 during 22 October 1983 to 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 the wheat. On each of three separate occasions, 18 locations in three bin strata were sampled twice for a total of 36 observations/sampling occasion/bin. Therefore, from Hagstrum *et al.* (1985) a total of 12 data sets were available. During July to December 1984, Hagstrum (1987) sampled two bins (4.3 or 6.4 m diameter) holding newly-harvested wheat (27- or 82-t capacity). On each sampling occasion, 11 sites in the top 1 m of each bin were probed twice with the grain trier to obtain a total of 22 0.5-kg samples. In the 27- and 82-t bins, sampling was done on 11 different occasions. Therefore, from Hagstrum (1987), a total of 22 data sets were available.

The presence and number of *C. ferrugineus* in each 0.5-kg sample (sample unit) were determined. A sample unit with one or more *C. ferrugineus* adults was scored as '1' and a unit without any adult was 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 sample mean ( $m$ ) and sample variance ( $s^2$ ). These sample statistics can be calculated manually (see Subramanyam and Hagstrum, 1995) or by a statistical software program (SAS Institute, 1988). For the 22 data sets,  $m$  ranged from 0.09 to 7.91 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 0.03 to 8.36 insects/sample unit, 0.029 to 338.56 and 0.028 to 0.944, respectively.

During bin sampling (Hagstrum, 1987), wheat moisture ranged from 10.2 to 13.0% and grain temperature from  $>1$  to  $32^{\circ}\text{C}$ . The grain samples were collected from harvest until the onset of cold temperatures; therefore, the insect densities reported here are representative of those occurring in bins under a range of environmental conditions.

### SAMPLING PLAN FOR ESTIMATING *C. FERRUGINEUS* DENSITY WITH A FIXED LEVEL OF PRECISION

#### Fixed precision level sequential sampling plan

When  $m$  is unknown, the number of sample units needed to estimate density at a fixed level of precision can be determined sequentially. Green (1970) proposed a sequential sampling approach in which sampling 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  $T_n$  = the cumulative number of insects in sample units,  $D$  is the precision expressed as a ratio of the standard error of the mean ( $SEM$ ) and  $m$  ( $SEM/m$ ) and  $A$  and  $b$  are estimated by regressing  $\ln(s^2)$  against  $\ln(m)$  (Taylor, 1961).  $A$  is a scaling factor related to the sample unit, and  $b$  is an indicator of the degree of insect aggregation.  $A$  is calculated as  $e^a$ , where  $e = 2.71828$  and  $a$  = y-intercept;  $b$  is the regression slope. After choosing a suitable  $D$ , Equation (1) is solved for different  $n$  values. A reasonable  $D$  is usually between 0.20 and 0.35 (Southwood, 1978; Hutchison *et al.*, 1988) because the observed precision is not fixed but stochastic. A plot of  $\ln(T_n)$  against  $\ln(n)$  gives a straight line (Green, 1970). A plot of  $T_n$  against  $n$  is nonlinear.

The linear regression of  $\ln(s^2)$  against  $\ln(m)$  based on the 22 data sets (Hagstrum, 1987) gave a y-intercept ( $a \pm SE$ ) of  $1.117 \pm 0.105$ , and a slope ( $b \pm SE$ ) of  $1.461 \pm 0.084$ . The y-intercept and slope were significantly greater ( $P < 0.01$ ) than 0 and 1, respectively. This indicated that *C. ferrugineus* adults were distributed in an aggregated fashion in the top 1 m of wheat stored in the bins. The scaling factor,  $A$ , was 3.056. Similar estimates were obtained for *C. ferrugineus* adults sampled with perforated probe traps placed just below the surface of farm-stored shelled corn (Subramanyam and Hagstrum, 1995, p. 156). To generate Green's (1970) fixed precision stop lines, Equation (1) was solved using a  $D$  of 0.25 or 0.35 and Taylor's estimates of  $A = 3.056$  and  $b = 1.461$ .

#### Fixed precision level sequential sampling plan validation

Figure 1 shows the fixed precision stop lines generated using Equation (1). The number of sample units required to cross the fixed precision stop lines decreased with an increase in insect density. In practice, to evaluate performance of the sampling plan, independent sample units are taken sequentially and the number of insects in them enumerated. The cumulative number of insects in sample units is plotted against the cumulative number of sample units with reference to the stop line. Counting stops when the fixed precision stop line is crossed.  $T_n/n$  at this point estimates  $m$ . The number of insects in each of the  $n$  sample units required to reach the stop line is used to calculate  $SEM$ .  $D$  is calculated as a ratio of  $SEM$  and  $m$ . This calculated  $D$  can then be compared with the fixed  $D$  used in Equation (1).

The performance of the fixed precision sampling plan was validated using the RVSP software. The 12 independent data sets (Hagstrum *et al.*, 1985), with a density range of 0.03 to 8.36 insects/sample unit, were resampled by RVSP for this analysis. Performance was evaluated by examining the actual  $n$  and  $D$  obtained from sequentially estimating

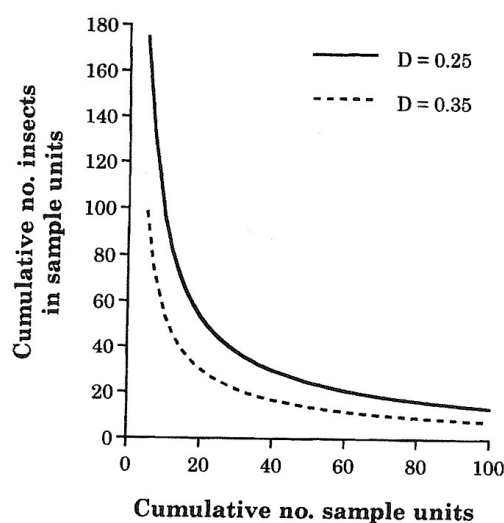


Fig. 1. Fixed-precision stop lines for sequential estimation of rusty grain beetle density. The precision ( $D$ ) is expressed as a ratio of standard error of the mean and mean.

*C. ferrugineus* densities (0.03 to 8.36 insects/sample unit) at specified precision levels of 0.25 and 0.35. To estimate 0.03 to 8.36 insects/sample unit at  $D = 0.25$ , 341 to 18 samples were required (Fig. 2). To estimate the same density range at  $D = 0.35$ , 181 to 10 samples

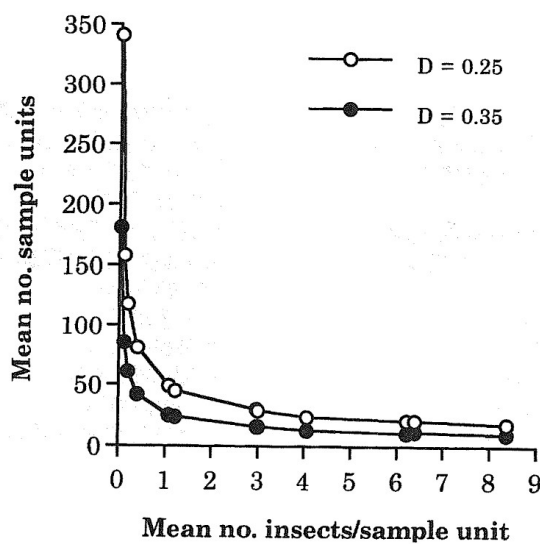


Fig. 2. The observed mean number of sample units (0.5-kg samples of wheat) required to estimate rusty grain beetle density between 0.03 and 8.36 insects/0.5 kg of wheat (sample unit). The RVSP program resampled each of the 12 independent data sets 500 times to determine the observed mean number of sample units. Therefore, each observed mean is the average of 500 simulations.

were required. About twice as many samples were required to estimate insect density at  $D = 0.25$  than at  $D = 0.35$ . The observed mean precision levels were worse than expected when estimating densities  $\leq 0.03$  or  $\geq 6.22$  (Fig. 3). The observed mean precision was better than expected when estimating densities between 0.11 and 1.22 insects/sample unit. For example, at  $D = 0.25$  and  $D = 0.35$ , the observed mean precision when estimating 0.11 to 1.22 insects/sample unit ranged from 0.22 to 0.18 and from 0.31 to 0.24, respectively. The observed precision when estimating densities of 2.97 and 3 insects/sample unit was worse than the expected precision. However, when estimating a density of 4.06 insects/sample unit, the observed precision was better than the expected precision. The precision was worse than expected (see Fig. 3) only when the variance predicted by Taylor's Power Law underestimated the actual (observed) variance (Table 1).

According to the United States Federal Grain Inspection Service (FGIS) standards, grain is classified as "infested" if a representative 1-kg grain sample contains a minimum of two live adults (Hagstrum and Flinn, 1992). Our sample unit was based on 0.5 kg of grain. Therefore, we chose one *C. ferrugineus* adult/sample unit as the economic threshold.

Based on Green's (1970) plan, a density of one insect/sample unit (1.08 insects/sample unit) can be estimated with 49 samples at  $D = 0.25$ , and with 25 samples at  $D = 0.35$  (Fig. 2). We recommend a fixed precision of 0.35 for estimating the economic threshold density of *C. ferrugineus* because the observed mean precision was 0.24 (Fig. 3). Except for a density of 4.06 insects/sample unit, the precision in estimating densities  $\leq 0.11$  or

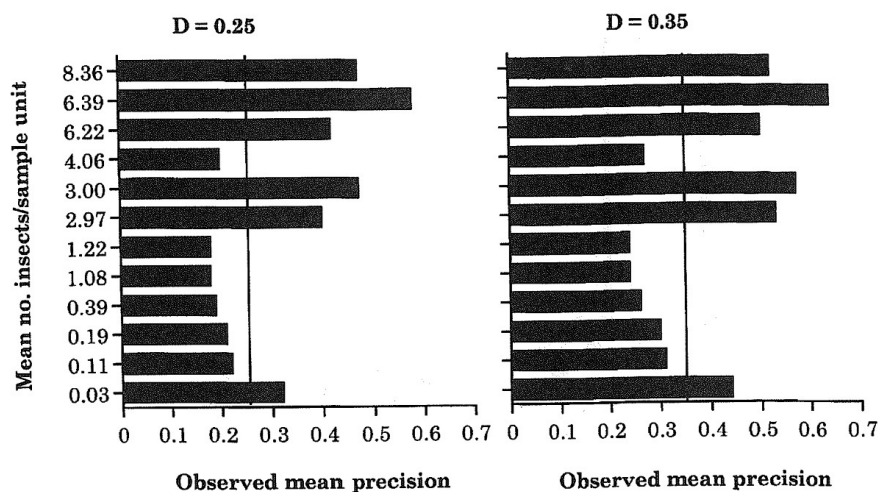


Fig. 3. The observed (actual) mean precision during estimation of rusty grain beetle density between 0.03 and 8.36 insects/0.5 kg of wheat (sample unit) at fixed precision levels ( $D$ ) of 0.25 and 0.35. The RVSP program resampled each of the 12 independent data sets 500 times to determine the observed mean precision. Therefore, each observed mean is the average of 500 simulations.

TABLE 1  
Observed sample mean ( $m$ ) and variance ( $s^2$ ) for the 12 independent data sets  
and the variance predicted by Taylor's Power Law ( $A = 3.056$  and  $b = 1.461$ )

Data set no.	$m^*$	$s^2$	Predicted $s^{2**}$
1	0.03	0.029	0.018
2	0.11	0.102	0.122
3	0.19	0.221	0.270
4	0.39	0.476	0.772
5	1.08	1.850	3.420
6	1.22	2.132	4.086
7	2.97	44.489	14.992
8	3.00	71.234	15.213
9	4.06	7.556	23.671
10	6.22	161.798	44.145
11	6.39	338.560	45.919
12	8.36	302.412	67.998

\*Expressed as mean number of live rusty grain beetles/sample unit (0.5 kg of wheat). Each mean is based on 36 observations.

\*\*The predicted variance was calculated as  $3.056 (m)^{1.461}$ , where  $m$  is the observed sample mean.

$\geq 1.22$  insects/sample unit often exceeded that desired. Furthermore, for estimating densities below 1.08 insects/sample unit, an unrealistically large number of samples was required. Our results suggest that the fixed precision sequential sampling plan is useful for estimating *C. ferrugineus* density at or near the economic threshold.

#### SAMPLING PLAN FOR CLASSIFYING *C. FERRUGINEUS* INFESTATION LEVEL RELATIVE TO AN ACTION THRESHOLD

Presence/absence or binomial sampling is a viable alternative when counting insects in sample units would be cumbersome or expensive (Binns and Nyrop, 1992). We developed a binomial sampling plan based on the sequential probability ratio test (SPRT) (Wald, 1947) to classify *C. ferrugineus* infestation levels relative to a threshold. 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 generally be established (Jones, 1994). Establishing this relationship is important for determining  $P(I)$  at the threshold density of one insect/sample unit.

#### Relationship between proportion of infested sample units and mean density

For the 12 data sets (Hagstrum *et al.*, 1985), the relationship between  $P(I)$  and  $m$  was established by regression techniques using TableCurve 2D (Anonymous, 1994). Figure 4 shows the nonlinear relationship between  $P(I)$  and  $m$ . The regression model fit the data well ( $R^2 = 0.907$ ). At the economic threshold density of one insect/sample unit, the  $P(I)$

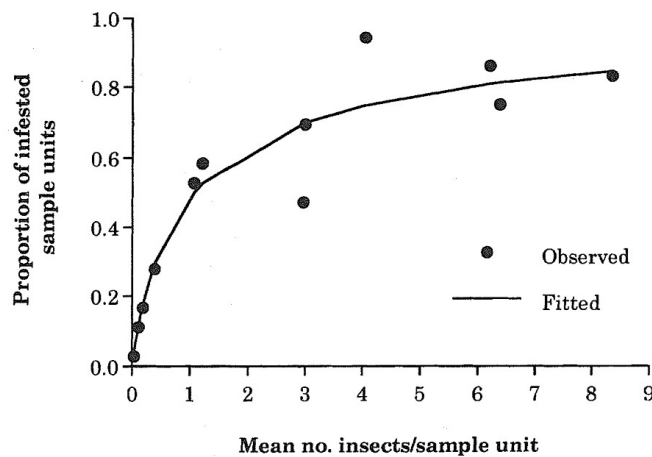


Fig. 4. Nonlinear relationship between proportion of sample units with one or more rusty grain beetles and mean density. The regression curve was fitted using TableCurve 2D, and the regression equation is described by:  $Y = 1.093(1 - 1/(1 + 2(1.093)^2 0.935X)^{0.5})$ ;  $n = 12$ ;  $R^2 = 0.907$ . At the United States Federal Grain Inspection Service 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.

was 0.485. For the 22 data sets (Hagstrum *et al.*, 1985), the  $P(I)$  against  $m$  regression gave similar results. Therefore, we used the 12 data sets for establishing the relationship between  $P(I)$  and  $m$ , and the 22 data sets for binomial SPRT sampling plan validation. Because of the variation in the empirical relationship between  $P(I)$  and  $m$ , and to take control action before the infestation level exceeds the economic threshold, we chose a  $P(I)$  of 0.43 or 43% as the action threshold (AT).

#### Binomial sequential sampling plan

To develop a sampling plan based on the binomial SPRT, information on four parameters is needed (Binns, 1994):  $p_1$  = the upper threshold,  $p_0$  = the lower threshold (usually a certain fraction of  $p_1$ ),  $\alpha$  = the probability of wrongly rejecting the null hypothesis that  $P(I) \leq p_0$  and  $\beta$  = the probability of wrongly rejecting the null hypothesis that  $P(I) \geq p_1$  (Jones, 1994).

We set  $p_1$  5% above the AT and  $p_0$  5% below the AT. Thus,  $p_1 = 0.48$  (equivalent to one insect/sample unit) and  $p_0 = 0.38$ . We set  $\alpha = \beta = 0.2$ . The error rates may be unequal (Waters, 1955) so the values used should be based on the acceptable risk of incorrect classification. For example, if  $\alpha = \beta = 0.2$ , then 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) through (5):

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

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

The intercept was calculated as:

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

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:

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

The upper and lower stop lines were generated by plotting  $T_U$  and  $T_L$  against  $n$  (Fig. 5). Data from independent samples, compared to these stop lines, could be used to determine if sampling should stop or proceed (Subramanyam and Hagstrum, 1995). Should data from the cumulative number of sample units with insects against the cumulative number of sample units cross either the upper or lower stop lines, sampling should be stopped. If the data falls within the stop lines, sampling should continue until the upper or lower stop line is crossed.

The two most important properties of the binomial SPRT sampling plan are the operating characteristic (OC) and the average sample number (ASN) functions. The procedures for calculating OC and ASN functions for binomial SPRT were given by Fowler and Lynch (1987). The OC function predicts the chance of correct classification; the ASN function tells us the average number of sample units required in the long run to reach a decision at each infestation level. The OC curve shows the probability of “not to inter-

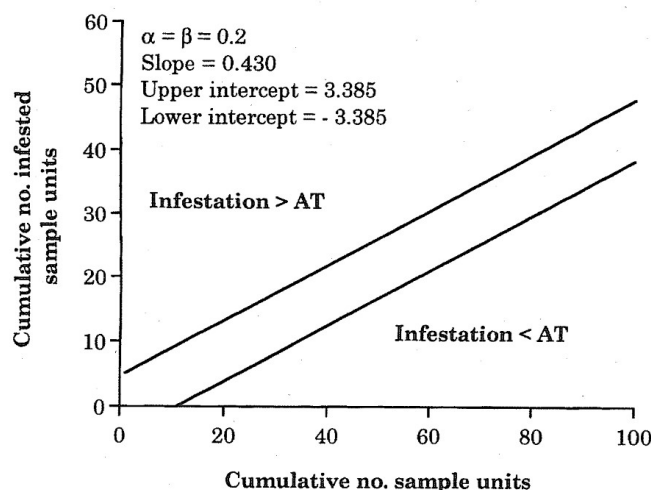


Fig. 5. Binomial sequential probability ratio test stop lines for classifying sample units infested with one or more rusty grain beetles with respect to the action threshold (AT) (see text for details).

vene" as a function of the infestation level. Typically, the OC function is near 1 when  $P(I) < p_0$ , and near 0 when  $P(I) > p_1$ . The OC function is 0.5 when  $P(I) = AT$  (for  $\alpha = \beta$ ). The ASN function increases if the lower and upper thresholds are closer together and if  $\alpha$  and  $\beta$  are set lower (Waters, 1955; Binns, 1994).

### Binomial sequential sampling plan validation

The performance of the 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. For each infestation level ( $P(I)$ ), the RVSP gave OC and ASN values, each ASN value an average of 500 iterations and each OC value the proportion of 500 resampling iterations terminating with a decision of not to treat (Naranjo and Hutchison, 1996). The OC or ASN values were plotted against corresponding  $P(I)$ , and a curve (Fig. 6) was fitted to the data points using TableCurve 2D (Anonymous, 1994).

At  $\alpha = \beta = 0.2$ , the OC function was near 1 when  $P(I)$  was  $\leq 0.3$ . At a  $P(I)$  of 0.38 ( $p_0$ ), the OC function was 0.851. At a  $P(I)$  of 0.48 ( $p_1$ ), the OC function was 0.189. At a  $P(I) > 0.48$ , the OC function decreased from 0.189 to 0. At a  $P(I)$  of 0.43 (AT), the OC function was 0.55. The OC curve at  $\alpha = \beta = 0.2$  indicated that the binomial SPRT sampling plan performed well in correctly classifying the *C. ferrugineus* infestation level with respect to the action threshold. Although the nominal errors were set at 0.2, the actual error rates generated by RVSP may be different. Actual error rates are determined from the OC curve (Fig. 6).  $1 - OC$  value at the lower threshold gives the actual  $\alpha$ ; OC value at the upper threshold gives the actual  $\beta$ . For the 22 data sets, actual  $\alpha$  and  $\beta$  were 0.149 and 0.189, respectively.

The ASN curve indicated that to classify  $P(I)$  between 0.045 and 0.909, an average of 7 to 51 sample units needed to be examined (Fig. 6). As expected, the ASN function was greatest near the action threshold. More sample units needed to be examined to reach a decision at  $P(I)$  between  $p_0$  and  $p_1$  than at  $P(I) < p_0$  or at  $P(I) > p_1$ .

The risks associated with using  $\alpha = \beta = 0.2$  are minimal, because the action threshold was set 5% below the economic threshold of 48% infested sample units (1 insect/sample unit). Furthermore, *C. ferrugineus* cannot feed on sound, dry grain; it can only develop on the germ of cracked or damaged grain (Sinha and Watters, 1985; White and Bell, 1990).

The ASN curve indicated that fewer sample units (~10) may be sufficient to classify very high and very low *C. ferrugineus* infestation levels. However, collection of sample units from a storage bin involves climbing up the bin, hauling sampling equipment into the bin, determining sampling locations, probing grain with the trier, bagging the sampled grain and climbing down the bin. Furthermore, because of inadequate bin head space, it is difficult, and sometimes impractical, to process sample units within the bin. In addition, the bin head space may get very hot during a warm day. Therefore, to avoid unnecessary sampling costs, we recommend collecting and bagging a large number of sample units from a bin. These sample units can be processed sequentially in the laboratory within 24 to 48 h of collection to separate live *C. ferrugineus* adults from the grain.

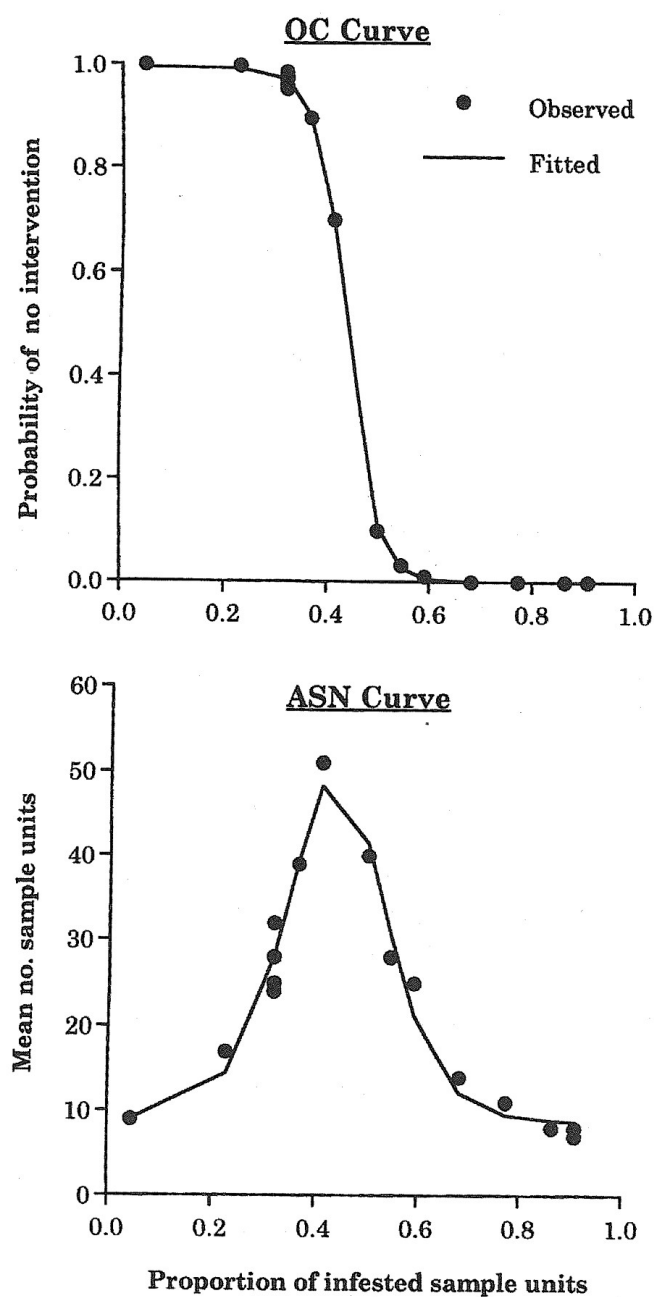


Fig. 6. Operating characteristic (OC) and average sample number (ASN) curves at  $\alpha = \beta = 0.2$  for the binomial sequential probability ratio test sampling plan. The RVSP program resampled each of the 22 independent data sets 500 times to determine the OC and ASN values. Therefore, each OC or ASN value is based on 500 iterations. The curves were fitted to data points using TableCurve 2D.

In Fig. 5, the lower stop line intersects the  $x$ -axis at 12 sample units. Therefore, the minimum sample size should be  $\geq 12$  sample units. The ASN curve indicated that a maximum of 51 sample units needed to be examined to reach a decision near the action threshold. Taking 51 sample units on a given day would be cumbersome. To reduce sampling costs, sampler fatigue and the risk of failing to find insects with  $\leq 12$  sample units, we suggest that at least 22 sample units be collected from a bin on a given day. The 22 0.5-kg wheat samples (sample units) should be taken with a 1.27-m grain trier from various locations in the top 1 m of wheat stored in a bin, following the sampling scheme outlined in Hagstrum *et al.* (1985) or Hagstrum (1987). The sample units should be processed sequentially in the laboratory, and information on the proportion of sample units with live *C. ferrugineus* adults compared to Fig. 5. If the proportion of sample units with one or more *C. ferrugineus* adults crosses the upper or lower stop line, sample processing should be stopped. If the upper stop line is crossed, indicating that infestation has exceed the action threshold, *C. ferrugineus* populations should be managed with a pesticide or a pesticide alternative. After a management action, the wheat should be resampled periodically (weekly or bimonthly) to determine the degree and duration of suppression of *C. ferrugineus* populations. If the lower stop line is crossed, indicating that infestation is below the action threshold, the wheat in bins should be resampled after 2 to 4 weeks depending on the grain temperature and moisture and the *C. ferrugineus* population growth rate at the existing environmental conditions. With 22 or fewer sample units, infestation levels of  $\leq 0.26$  or  $\geq 0.60$  can be classified with respect to the action threshold (see ASN curve in Fig. 6). Thus, to classify infestation levels between 0.26 and 0.60, more than 22 sample units are required. If a decision is not reached after processing all 22 sample units, it is apparent that the infestation level is between 0.26 and 0.60. If more than 22 sample units are required, they should be collected after a few days or a week. These additional 22 sample units should be collected and processed and the sampling information then interpreted as outlined above.

The development and validation of sequential sampling plans for estimating density and classifying infestation level of *C. ferrugineus* adults in 0.5-kg samples of wheat have been illustrated here for the first time. The methods proposed here are useful for accurately estimating *C. ferrugineus* insect density and infestation level, which are important for evaluating the effectiveness of a control measure.

#### ACKNOWLEDGEMENT

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