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# Calibration of sampling techniques and determination of sample size for the estimation of egg and larval populations of *Helicoverpa* spp. (Lepidoptera: Noctuidae) on irrigated soybean

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**Abstract** Informed spray decisions require accurate assessments of the target pest's density. Currently, no advice is provided to farmers on the best method for sampling soybean for insect pests, although spray thresholds for *Helicoverpa* larvae are provided. This article describes the results of a trial designed to calibrate relative sampling techniques for *Helicoverpa* larvae; visual inspection of plants *in situ* in the field, beat cloth, sweep net and D-vac sampling were compared with an absolute measure of population density. The absolute measure was derived from the bagging and removal of whole plants in the field, followed by subsequent examination in the laboratory. Analysis of the distribution of *Helicoverpa* larvae collected by the different samples was then used to calculate the number of samples required to determine whether the economic threshold had been reached to different levels of certainty and accuracy. Significant relationships were detected between all the relative sampling techniques and the absolute, suggesting that all could be used to estimate field populations. However, due to the high variance and therefore increased sample sizes required, or the length of time taken to collect samples, only beat-cloth sampling appeared to offer a realistic method for farmers in the field. The results also suggest that the current best practice of sampling six locations per crop provides an adequate assessment of the field populations at the currently accepted threshold level of 6 larvae $m^{-2}$. However, if the economic spray was reduced, the number of samples required to determine an accurate population estimate would increase dramatically.

**Key words** absolute sampling, beat cloth, D-vac, sweep net, visual sampling.

## INTRODUCTION

Informed spray decisions require accurate assessments of the population density of the target pest (Dent 1991). Obtaining an absolute measure of insect pest populations is invariably expensive and time-consuming. Therefore, treatment decisions are normally based on population estimates derived from relative sampling techniques (Kogan & Pitre 1980). Effective relative sampling can still be time-consuming and therefore costly. In crops such as cotton, where the value of the crop is high, investment in intensive sampling is justified, while in grain crops such as soybean, where the margins are low, sampling for insect populations needs to be simple and time-effective.

The main insect pests of irrigated soybean in southern New South Wales (NSW) are *Helicoverpa armigera* (Hübner) and *Helicoverpa punctigera* (Wallengren), which are targeted by up to four insecticide applications each season (SJ Duffield unpubl. data 2003). The current spray threshold for *Helicoverpa* larval control issued by NSW Department of Primary Industries is 3–6 larvae $m^{-2}$, depending on the drought stress of the crop (Colton *et al*. 1995). However, no advice is given on the most appropriate technique of sampling the crop to determine the size of the *Helicoverpa* population. Current 'best farm practice' is to place an empty fertiliser bag between the rows and shake the adjacent crop. This is then repeated in up to six locations around the field (SJ Duffield unpubl. data 2003).

There are several methods for sampling insect populations in soybean, including visual assessment, beat cloth, sweep net and D-vac sampling, that have been used commercially around the world (Kogan & Pitre 1980). All these techniques can be regarded as being relative sampling techniques (Kogan & Pitre 1980), as they do not record the whole or absolute population.

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The first component of any sampling plan to be used in decision making against thresholds based on absolute population densities is to calibrate the relative sampling technique to the absolute population in the field. The second stage is then to determine the number of samples to be taken. The number of samples required is dependent on the accuracy of the estimate required and the variability of the pest population (Dent 1991). The number of samples taken is often a trade-off between the time available and the accuracy of the population estimate obtained (Cullen *et al*. 2000).

This article describes a trial designed to calibrate different relative sampling techniques for *Helicoverpa* spp. on irrigated soybean against an absolute measure. We then provide an analysis of the distribution of *Helicoverpa* populations recorded by the different techniques to provide information on the number of samples required to achieve different levels of accuracy. The objective was to provide advice to farmers on the most appropriate sampling method, and the number of samples required to enable informed spray decisions based on the current advised threshold.

## MATERIALS AND METHODS

The trial was conducted in January–March 1999 in fields of commercially grown irrigated soybean in the Murrumbidgee and Coleambally Irrigation Areas of the Riverina region of southern NSW, Australia. The relative sampling techniques evaluated were visual inspection, sweep net, beat cloth and D-vac. Whole-plant destructive sampling was used to obtain an absolute measure of populations.

Absolute (ABS): A paper sack was placed over 0.5 m of row. The stems were cut at ground level, removed and the sack sealed. Two beat cloths placed each side of the sampling area were used to detect and record any *Helicoverpa* larvae that fell off the crop during the 'bagging'. The collected samples were stored in a cold room at 4°C and examined within 24 h of collection. Each plant was inspected in the laboratory and all *Helicoverpa* life stages identified.

Visual inspection (VIS): A 0.5 m section of row was selected. Each plant within the 0.5 m was carefully examined from bottom to top *in situ*. All external plant surfaces including the terminal were examined. The adjacent ground surface was also inspected for larvae that had fallen from the crop during the inspection.

Sweep net (SW): Ten sweeps were performed lengthways along a row (approximately 10 m) using a 0.38 m diameter sweep net. The contents of each sample were transferred to a cloth sample bag which was sealed. The sample was frozen and examined in the laboratory.

Beat cloth (BC): A 1 m section of row was selected and a 1 m beat cloth unrolled in the adjacent furrow. The crop was bent over the beat cloth and vigorously struck with a stick several times. All insects that fell on the beat cloth were counted in the field.

D-vac (DV). A Weed Eater Super Blowervac (Model DV1800) with a 12 cm diameter suction area was adapted by attaching a nylon mesh collection bag to the nozzle. We sampled 5 m of row with the D-vac on full throttle. Each individual plant was sampled from bottom to top. The collection bag was removed with the machine still on full throttle and placed into a sample bag. The samples were frozen and subsequently examined in the laboratory.

The length of row used for each sampling technique was chosen to ensure that comparable numbers of larvae were sampled by each method. We sampled 10 sites at three different growth stages: vegetative (V4-9) (Fehr *et al*. 1971), flowering (R2-3) and pod fill (R5-6). At each site, six individual rows were selected for sampling, each separated by four unsampled rows. Each of the sampling methods was conducted along each of the selected rows, separated by a minimum of 20 m. The order the samples were taken in along the row was randomised. The time taken to collect and count the insects by each method was recorded for both the field and laboratory component.

The mean numbers (±1 SE) of *Helicoverpa* eggs and larvae collected using each technique were calculated at vegetative, flowering and podding growth stages (Table 1). Differences in numbers recorded during the different growth stages were tested by ANOVA ($\log_{10}(n+1)$ transformed to ensure equality of variance). Additionally, the overall mean and its relative variation (RV) (Southwood 1978) for each sampling technique were calculated as well as a correction factor (CF) derived by dividing the absolute count by the overall mean for each method, to transform visual, beat cloth, D-vac and sweep net into equivalent absolute values (Pitre *et al*. 1987).

In a preliminary analysis, we used multiple regression analysis to determine the relationship between mean absolute count (the dependent variable), initially against the independent variables of count and growth stage for each of the relative sampling methods separately. Counts were $\log_{10}(n+1)$ transformed. For all groups, crop growth stage was excluded from further analysis, as it had a consistently non-significant effect on model fit. Hence, in further analyses a simple regression relationship between mean absolute count and count of relative sampling method was determined.

We followed the method of Cullen *et al*. (2000) to determine the sample size required to fulfil specific requirements regarding the accuracy of the population estimate for the current economic threshold. This analysis was conducted for the total number of *Helicoverpa* larvae using the currently used spray threshold of 6 larvae $m^{-2}$, which is currently used most commonly with the beat cloth method.

An equation for estimating sample size was developed by Karandinos (1976) and modified by Wilson (1985) to incorporate Taylor's Power Law (Taylor 1961) as:

$$n = t^2_{\alpha/2} D^{-2} a \theta^{b-2}$$

where $n$ = the number of samples required, and $t_{\alpha/2}{}^2$ = standard two-tailed normal variate (commercially this equates to the percentage certainty of the decision being correct), which we varied from 10% to 99%; $\theta$ = treatment threshold. For beat cloth samples, we used the commercially recognised threshold of 6. Thresholds for the other sampling methods were esti-

***Table 1*** Summary statistics (mean, SE and *n*) from ANOVA to detect differences between growth stages

| Insect and growth stage | Sampling technique | | | | |
|---|---|---|---|---|---|
| | Absolute (0.5 m) | Visual (0.5 m) | Beat cloth (1.0 m) | D-vac (5.0 m) | Sweep (10.0 m) |
| *Helicoverpa* eggs | | | | | |
| Vegetative | 3.1 ± 1.1 (10) | 1.6 ± 0.5 (10) | | | |
| Flowering | 21.6 ± 10.45 (10) | 19.3 ± 5.9 (10) | | | |
| Podding | 30.6 ± 4.6 (10) | 31.3 ± 6.4 (10) | | | |
| | $F = 14.6$ | $F = 28.8$ | | | |
| | $P < 0.0001$ | $P < 0.0001$ | | | |
| Overall mean | 18.43 ± 4.26 | 17.4 ± 3.6 | | | |
| Correction factor | | 1.06 | | | |
| Relative variation | 23.1 | 20.6 | | | |
| I–III instar larvae | | | | | |
| Vegetative | 9.0 ± 3.20 (10) | 3.5 ± 1.10 (10) | 4.4 ± 1.36 (10) | 14.4 ± 9.43 (8) | 5.4 ± 2.74 (10) |
| Flowering | 6.4 ± 1.77 (10) | 3.2 ± 1.09 (10) | 4.9 ± 1.83 (10) | 2.0 ± 0.75 (10) | 0.7 ± 0.26 (10) |
| Podding | 6.0 ± 1.84 (10) | 5.3 ± 2.37 (10) | 5.4 ± 1.81 (10) | 1.8 ± 0.80 (5) | 4.4 ± 1.10 (10) |
| | $F = 0.049$ | $F = 0.398$ | $F = 0.145$ | $F = 1.723$ | $F = 4.456$ |
| | $P = 0.95$ | $P = 0.68$ | $P = 0.87$ | $P = 0.20$ | $P = 0.021$ |
| Overall mean | 7.3 ± 1.34 | 4.0 ± 0.92 | 4.9 ± 0.94 | 6.3 ± 3.40 | 3.5 ± 1.03 |
| Correction factor | | 1.83 | 1.49 | 1.17 | 2.09 |
| Relative variation | 18.8 | 23.1 | 19.2 | 54.4 | 29.4 |
| IV–VI instar larvae | | | | | |
| Vegetative | 4.9 ± 2.10 (10) | 3.2 ± 1.24 (10) | 1.9 ± 0.71 (10) | 7.1 ± 3.24 (8) | 2.5 ± 0.93 (10) |
| Flowering | 3.8 ± 1.36 (10) | 3.1 ± 0.80 (10) | 4.0 ± 1.26 (10) | 1.3 ± 0.50 (10) | 1.7 ± 0.54 (10) |
| Podding | 3.5 ± 0.67 (10) | 3.5 ± 0.99 (10) | 3.7 ± 0.94 (10) | 0.8 ± 0.20 (5) | 3.6 ± 0.67 (10) |
| | $F = 0.074$ | $F = 0.243$ | $F = 1.406$ | $F = 3.709$ | $F = 2.669$ |
| | $P = 0.93$ | $P = 0.79$ | $P = 0.26$ | $P = 0.043$ | $P = 0.088$ |
| Overall mean | 4.1 ± 0.85 | 3.3 ± 0.57 | 3.2 ± 0.58 | 3.2 ± 1.26 | 2.6 ± 0.43 |
| Correction factor | | 1.24 | 1.27 | 1.26 | 1.56 |
| Relative variation | 21.0 | 17.4 | 18.1 | 39.1 | 16.5 |
| Total *Helicoverpa* larvae | | | | | |
| Vegetative | 13.9 ± 5.20 (10) | 6.7 ± 2.20 (10) | 6.3 ± 2.00 (10) | 21.5 ± 11.71 (8) | 7.9 ± 3.60 (10) |
| Flowering | 10.2 ± 2.47 (10) | 6.3 ± 1.70 (10) | 8.9 ± 2.50 (10) | 3.3 ± 1.10 (10) | 2.4 ± 0.70 (10) |
| Podding | 9.5 ± 2.33 (10) | 8.8 ± 3.10 (10) | 9.1 ± 2.42 (10) | 2.6 ± 0.90 (5) | 8.0 ± 1.60 (10) |
| | $F = 0.500$ | $F = 0.400$ | $F = 0.600$ | $F = 2.200$ | $F = 3.745$ |
| | $P = 0.63$ | $P = 0.68$ | $P = 0.54$ | $P = 0.13$ | $P = 0.037$ |
| Overall mean | 11.2 ± 2.03 | 7.3 ± 1.34 | 8.1 ± 1.31 | 9.5 ± 4.35 | 6.1 ± 1.38 |
| Correction factor | | 1.54 | 1.38 | 1.18 | 1.84 |
| Relative variation | 18.1 | 18.4 | 16.2 | 45.8 | 22.6 |

mated using the regression relationships obtained (Table 2) and were 8.6 for a 0.5 m absolute count, 5.6 for a 0.5 m visual count, 5.9 for a 5 m D-vac sample and 4.5 for a 10 m sweep. Hence, the thresholds were considered to be equivalent. $D$ is a fixed proportion of the treatment threshold representing the range of accuracy around the population mean. The value of $\theta_{adj}$ is the treatment threshold ($\theta$) adjusted by a percentage value. $D$ can be regarded as the commercial confidence level, as $D$ decreases the confidence level decreases and the number of samples required necessary for an accurate treatment decision increases. We set this value initially at 10%. We derived $a$ and $b$, the Taylor's coefficients, for each sampling technique by using the mean–variance relationships. Means and variances were calculated from the six row counts taken on each sampling occasion (10 sites by 3 growth stages), resulting in a single mean–variance pair being included in the regression for each site, growth-stage combination.

## RESULTS

The mean numbers of *Helicoverpa* eggs and larvae collected using each technique were calculated at vegetative, flowering and podding growth stages (Table 1). Significantly ($P < 0.001$) different numbers of *Helicoverpa* eggs were recorded in the different plant growth stages for both the absolute and visual techniques, with numbers increasing as the crop developed. This trend was not reflected in differences in the numbers of *Helicoverpa* larvae recorded at the different growth stages. Significant differences ($P < 0.05$) were recorded for I–III instar larvae and total larvae by the sweep net, although this reflected a reduction in numbers at flowering, and for IV–VI larvae for the D-vac.

The RV for *Helicoverpa* eggs ranged from 20.6 for visual sampling to 23.1 for the absolute technique. For *Helicoverpa* larvae, the D-vac samples tended to have the highest RV values

(Table 1). The CF for *Helicoverpa* larvae ranged from 1.18 to 1.84, indicating that the different techniques sampled similar numbers of individuals. However, the area of crop sampled by the various techniques differed considerably (0.5–10.0 m), indicating that the efficiency in terms of the individuals caught per unit of crop varied widely.

We detected a significant regression relationship ($P < 0.001$) between the visual counts and the absolute for *Helicoverpa* eggs, with an $R^2$ of 74.7% (Table 2). All sampling techniques showed significant ($P < 0.001$) relationships for I–III, IV–VI and total *Helicoverpa* larvae. The highest level of significance and highest $R^2$ values were recorded by the beat cloth and visual sampling methods. The time taken to collect and count samples using the different techniques is shown in Table 3. The total overall time was lowest for beat cloth samples, which took between 2.8 and 3.3 min per sample. The absolute method took the longest, ranging from 24.1 to 33.7 min, primarily due to the length of time taken in the laboratory to examine the sample.

***Table 2*** Summary of regression analysis of relative sampling technique counts against the absolute sample count. Log-log transformed

| Independent | Equation | $R^2$ (%) | Probability |
|---|---|---|---|
| *Helicoverpa* eggs | | | |
| Visual | 0.827V + 0.187 | 74.7 | <0.001 |
| Beat cloth | | | |
| D-vac | | | |
| Sweep | | | |
| I–III instar larvae | | | |
| Visual | 0.762V + 0.337 | 49.4 | <0.001 |
| Beat cloth | 0.652B + 0.38 | 58.5 | <0.001 |
| D-vac | 0.452D + 0.491 | 35.4 | 0.003 |
| Sweep | 0.550S + 0.518 | 35.5 | 0.001 |
| IV–VI instar larvae | | | |
| Visual | 0.653V + 0.252 | 51.4 | <0.001 |
| Beat cloth | 0.668B + 0.248 | 51.0 | <0.001 |
| D-vac | 0.560D + 0.311 | 33.5 | 0.004 |
| Sweep | 0.706S + 0.252 | 42.5 | <0.001 |
| Total *Helicovpera* larvae | | | |
| Visual | 0.808V + 0.318 | 68.0 | <0.001 |
| Beat cloth | 0.665B + 0.420 | 63.1 | <0.001 |
| D-vac | 0.543D + 0.526 | 44.2 | 0.001 |
| Sweep | 0.659S + 0.493 | 47.9 | 0.001 |

The method of Cullen *et al*. (2000) was followed to determine the sample size required to fulfil specific requirements regarding the accuracy of the population estimate for the current economic threshold. Table 4 shows the number of samples required for each sampling technique to achieve different levels of certainty ($t_{\omega/2}^2$) when the level of accuracy required ($D$) was set at 10%. To achieve an 80% level of certainty the number of samples required varied from 52 (D-vac) to 22 (absolute). The numbers of samples required for different levels of certainty ($t_{\omega/2}^2$) for the beat cloth sampling of *Helicoverpa* larvae were determined over a range of required accuracy ($D$) (Table 5). If the level of accuracy was reduced from 10% (6 larvae ± 0.6) to 20% (6 larvae ± 1.2), the number of samples required to achieve an 80% level of certainty declined from 25 to 7.

The number of samples required by beat cloth sampling to estimate populations for different hypothetical thresholds was also determined (Table 6). If the treatment threshold was 9 larvae $m^{-2}$ the number of samples required to reach 80% certainty and 20% accuracy dropped from 7 to 5. However, if the treatment threshold was 3 larvae $m^{-2}$ the number of samples required would rise to 13.

## DISCUSSION

Our results indicate that all the sampling techniques that we evaluated have potential for use for sampling *Helicoverpa* larvae in irrigated soybean, as we identified significant relationships between the different relative sampling techniques and the absolute measure. An RV of less than 25 is generally regarded as being acceptable for most sampling program (Southwood 1978), and the RV of most of the techniques, excluding the D-vac, fell within this range. The RV values for *Helicoverpa* larvae recorded in this study are similar to those recorded for *Heliothis zea* in soybean in the southern United States (Kogan & Pitre 1980), which also identified D-vac as a poor method of sampling. The parameters *a* and *b* for Taylor's Power Law were within the range of those found in previous studies of arthropods (Taylor 1961).

The distribution of *Helicoverpa* eggs on soybean changes as the crops develops, with a greater proportion laid in the lower portion of the crop during the podding stage (Duffield & Chapple 2001). This, however, is not reflected in any change in the relative effectiveness of the sampling techniques, as we

***Table 3*** Sampling times (min) for each method at three growth stages. Sweep net, D-vac and absolute times were broken down into field and laboratory times

| Growth stage | Sampling technique | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Absolute | | | Visual | Beat cloth | D-vac | | | Sweep net | | |
| | Field | Laboratory | Total | – field | – field | Field | Laboratory | Total | Field | Laboratory | Total |
| Vegetative | 3.6 | 26.3 | 29.9 | 5.8 | 3.1 | 2.7 | 8.8 | 11.5 | 2.4 | 4.2 | 6.6 |
| Flowering | 4.4 | 29.3 | 33.7 | 9.9 | 3.3 | 2.4 | 4.4 | 6.8 | 3.4 | 2.1 | 5.5 |
| Podding | 4.1 | 20.0 | 24.1 | 9.8 | 2.8 | 1.9 | 3.0 | 5.0 | 2.9 | 2.0 | 4.9 |
| Mean | | | 29.2 | 8.5 | 3.1 | | | 7.7 | | | 5.6 |

***Table 4*** Required sample size for the different sampling techniques (after Cullen *et al*. 2000) for different levels of certainty for a population of six larvae per row metre as measured by a beat cloth. Accuracy set at 10%

| Level of certainty | Sampling technique | | | | |
|---|---|---|---|---|---|
| | Absolute | Visual | Beat cloth | D-vac | Sweep net |
| 99 | 96 | 134 | 109 | 225 | 159 |
| 90 | 37 | 51 | 42 | 86 | 61 |
| 80 | 22 | 31 | 25 | 52 | 37 |
| 70 | 15 | 20 | 16 | 34 | 24 |
| 60 | 10 | 13 | 11 | 22 | 16 |
| 50 | 6 | 9 | 7 | 14 | 10 |
| 40 | 4 | 5 | 5 | 9 | 6 |
| 30 | 2 | 3 | 3 | 5 | 4 |
| 20 | 1 | 2 | 1 | 2 | 2 |
| 10 | 1 | 1 | 1 | 1 | 1 |
| Equivalent threshold | 8.6 | 5.6 | 6.0 | 5.9 | 4.5 |

***Table 5*** Required sample size for beat cloth sampling to reach different levels of accuracy (population density of six larvae per row metre)

| Level of certainty | Required level of accuracy (%) | | |
|---|---|---|---|
| | 10 | 15 | 20 |
| 99 | 109 | 49 | 28 |
| 90 | 42 | 19 | 11 |
| 80 | 25 | 11 | 7 |
| 70 | 16 | 8 | 4 |
| 60 | 11 | 5 | 3 |
| 50 | 7 | 3 | 3 |
| 40 | 5 | 2 | 2 |
| 30 | 3 | 1 | 1 |
| 20 | 1 | 1 | 1 |
| 10 | 1 | 1 | 1 |

***Table 6*** Required sample size for beat cloth sampling to determine different threshold densities with an accuracy set at 20%

| Level of certainty | Threshold density (larvae per row metre) | | |
|---|---|---|---|
| | 3 | 6 | 9 |
| 99 | 56 | 28 | 18 |
| 90 | 22 | 11 | 7 |
| 80 | 13 | 7 | 5 |
| 70 | 9 | 4 | 3 |
| 60 | 6 | 3 | 2 |
| 50 | 4 | 2 | 2 |
| 40 | 3 | 2 | 1 |
| 30 | 2 | 1 | 1 |
| 20 | 1 | 1 | 1 |
| 10 | 1 | 1 | 1 |

could detect no crop growth stage effect, suggesting that a single method could be used for all growth stages.

Absolute samples provide the most accurate assessment of both egg and larval density. This sampling method was the most systematic method used, and would require fewer samples to be taken compared with a beat cloth for larval sampling in order to reach the same level of accuracy and certainty. However, due to the excessive time it takes to collect the samples, absolute sampling would not be a viable option for farmers. Visual sampling is the only viable technique for the sampling of *Helicoverpa* eggs on soybean. However, no economic threshold has been developed for *Helicoverpa* eggs on soybean, so it is unlikely that the sampling of eggs would provide useful information when making spray decisions at this stage.

The results in terms of the strength of relationship between the relative and absolute, relative variation and the required time to take the samples suggested that beat cloth samples offered the best form of sampling soybean for *Helicoverpa* larvae under the conditions and varieties grown in Australia.

Our study indicates that the current best practice of sampling six locations provides a 75–80% certainty that populations are at the threshold density of 6 larvae $m^{-2}$ to an accuracy of 20% or ±1 larvae $m^{-2}$. Such a level of certainty and accuracy is likely to be acceptable to many growers making spray decisions in the field. However, if the economic spray threshold was lower than the 6 larvae $m^{-2}$ currently used, this would have considerable implications in terms of how the crop is sampled due to the sensitivity of the number of samples required to accurately determine a lower threshold density. As the spray threshold declines, the number of samples required to determine an accurate estimate increases dramatically.

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