Evaluation and Calibration of Sampling Methods Used to Estimate Abundance of Alfalfa Weevil Larvae (Coleoptera: Curculionidae)[1]

John M. Luna and F. William Ravlin

Department of Entomology, Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

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ABSTRACT Three sampling methods for estimating abundance of alfalfa weevil larvae (*Hypera postica* Gyllenhal) were evaluated for both accuracy and precision. A plastic bag/Tullgren funnel method collected significantly fewer second, third, fourth instar and total larvae than did the bucket-shake method. Calibration equations were developed to convert intensity estimates (alfalfa weevil larvae per alfalfa stem) obtained from the plastic bag/Tullgren funnel method to bucket-shake estimates. Sweep-net sampling produced significantly lower coefficients of variation than both bucket-shake and plastic bag/Tullgren funnel sampling methods, however mean estimates of larval abundance obtained from sweep-net sampling could be reliably converted to bucket-shake estimates for only fourth instar larvae.

KEY WORDS Alfalfa weevil, *Hypera postica,* sampling methods.

Several sampling techniques are commonly used by researchers and integrated pest management (IPM) practitioners to estimate abundance of alfalfa weevil larvae (*Hypera postica* Gyllenhal) in alfalfa. Selection of a particular technique should be based on the sampling objectives and include considerations of accuracy, precision and cost. Estimates of absolute larval density (larvae per unit area) are usually derived by extracting larvae from alfalfa stems clipped from measured quadrat samples. Roberts et al. (1979) compared two systems for exacting larvae, a modified Tullgren funnel, and a preservative, wash, hand sorting method. Statistically significant differences between mean estimates of larval densities using the two methods were reported for 20% of the 108 paired means in the experiment, with each method giving higher estimates in about half of the cases where the estimates differed. Roberts et al. concluded the modified Tullgren funnel is an acceptable technique for extracting weevil larvae. Smart et al. (1985) reported a study comparing Tullgren funnel extraction with manual examination of foliage and claimed the Tullgren funnel was an "adequate" method, however no supporting data were presented. Guppy et al. (1975) used a modified Tullgren funnel to estimate larval intensities for development of fixed-precision sampling plans for AW larvae, however no evaluation was made of the sampling method.

Another sampling method, in which alfalfa stems are pulled into a bucket and shaken to dislodge weevil larvae (henceforth called the "bucket -shake"

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technique), has been widely used in IPM programs in several states (Wedberg 1977, Gesell et al. 1984, Luna 1985). This method is a modification of the pan technique (Blickenstaff and Huggans 1969) in which alfalfa stems are bent over a shallow pan and shaken to dislodge larvae. The bucket-shake method usually involves collecting from 10 to 30 alfalfa stems into a bouquet before shaking, and is presumably more efficient than the pan method because the stems can be beaten vigorously against the side of the bucket to dislodge larvae contained from the foliage. Legg et al. (1985) compared larval estimates obtained from bucket-shake sampling with total larval estimates obtained by visually inspecting the alfalfa tips after they had been shaken in the bucket and adding these to the bucket-shake estimates. Although no significant differences were detected in mean estimates, the bucket-shake method underestimated absolute stem intensities by 0.04 to 0.08 larvae per stem. These authors considered this error so small as to seldom be of consequence. In a later study, Barney and Legg (1989) reported conversion factors for converting bucket-shake estimates of larval intensity to absolute intensity for second to fourth instars. Barney and Legg (1987) examined the accuracy of using a single 30-stem sample for the bucket shake technique to make pest management decisions. These authors concluded that a single 30-stem sample gave a poor estimate of population densities and that multiple samples gave somewhat better estimates.

Sweep-net sampling has been widely used as a sampling method for alfalfa weevil larvae (Blickenstaff 1966, Armbrust et al. 1969), however several authors have demonstrated the inadequacies of the sweep-net for estimating absolute larval densities (Blickenstaff and Huggans 1969, Cothran and Summers 1972). According to Cothran and Summers (1972), relative density estimates obtained from sweep-net sampling cannot be converted to absolute density estimates, and suggest that many population studies utilizing the sweep-net "fail significantly to describe accurately the actual population pattern."

This study was initiated to evaluate and compare three sampling methods for estimating abundance of alfalfa weevil larvae (bucket-shake, sweep-net, and a plastic bag/Tullgren funnel), and to develop equations to convert density estimates obtained from one method to another.

Materials and Methods

Data were collected from seven commercial alfalfa fields in three Virginia counties (Bedford, Rockbridge and Augusta) in 1985. On each sample date, 8 samples per field were taken using the Tullgren funnel and the bucket-shake methods, and 10 samples were taken using the sweep-net. Samples for each method were taken within 10 m of each other. Data were collected from 14 field-dates. Alfalfa stem lengths ranged from 12 to 55 cm during the experiment, and alfalfa weevil intensities ranged from 0.18 to 1.54 total larvae per stem (estimated using the bucket-shake method).

Plastic bag/Tullgren Funnel Extraction Method. As mentioned earlier, absolute *density* estimates involve extraction of larvae from foliage within a measured quadrat. In an effort to standardize sampling methods for alfalfa weevil larvae, Armbrust et al. (1969), suggested extracting larvae from alfalfa stems randomly collected within a research plot. This procedure, however, generates estimates of weevil *intensity* per stem, rather than absolute density per unit area of land. Conversion of larval intensity per stem to absolute density can be made by multiplying larval intensity by an estimate of the density of alfalfa stems per unit area, if this latter value is known. Larval intensity per alfalfa stem was estimated for this sampling method comparison study for two reasons: larval intensity estimates are the estimates derived from bucket-shake sampling used in IPM programs, and larval intensity per stem is directly proportional to the absolute density, although this proportion is variable.

Samples for the plastic bag/Tullgren funnel method were obtained by selecting 20 alfalfa stems at each sample site. Stems were pulled (severing them from the base of the alfalfa plant) and placed tip-first into a 20 by 40 cm plastic bag. Stems to be sampled were selected using a "systematic sampling" method (Legg et al. 1985) in which the sampler took 2 paces (ca 1.5 m) from a randomly selected starting point, reached down into the foliage without looking and selected the first stem contacted. According to Legg et al. (1985), systematic sampling for weevil larvae gives comparable results to simple random sampling using totally randomly selected sample sites, but is much easier and faster to use than simple random sampling.

Plastic bags from each field were placed in an ice chest until returning to the laboratory for processing. Samples were placed in modified Tullgren funnels similar to those used by Roberts et al. (1979) and larvae were collected in jars containing 70% ethanol. Samples remained in the funnels for approximately 24 hours. Larvae were sorted to instar based on size and color, and counted. This sampling method will henceforth be referred to as the "Tullgren funnel" method.

Bucket-Shake Method. Ten alfalfa stems per sample were randomly selected and removed (using the same procedures as described above for the Tullgren funnel method) and placed into a 30 cm dia, 19 liter bucket. The bottoms of the stems were gathered and the stems were shaken vigorously for approximately 10 seconds. A clipboard was placed over the top of the bucket while shaking to minimize loss of larvae. Larvae were poured into a jar containing 70% ethanol and later counted in the laboratory.

Sweep-net Sampling. A 37 cm dia. muslin sweep-net was used with a pendulum sweeping motion (Cothran et al. 1975) and 10 sweeps per sample. Sweep-net contents were poured into 70% ethanol and later counted in the laboratory.

Analytical Procedures. Bucket-shake and Tullgren funnel sampling methods were compared using the Student's t-test for mean estimates of alfalfa weevil larvae by instar for each field and date in the experiment (Steel and Torrie 1960). Least squares linear regression was used to develop calibration models to convert mean estimates obtained from Tullgren and sweep-net sampling to mean estimates obtained from bucket-shake sampling (SAS, 1985). Regressions were conducted using sample means for each field and sample date on which sampling was conducted. Separate regressions were performed for each of the four larval instars and for total larvae. In these analyses, mean estimates from the bucket-shake method were used as the dependent variable and mean estimates from the other two sampling methods were the independent variables (Luna et al. 1982).

Precision, the second criterion for evaluating sampling methods, was estimated by calculating the coefficient of variation (CV) (Steele and Torrie 1960) where:

$$CV= 100 \quad \frac{s^2}{\overline{X}}$$

where s^2 = variance estimate and $\overline{X}$ = mean estimate. Coefficients of variation were calculated for each sampling method and larval instar for each field and sample date. Average CV's for each sampling method and instar were computed and compared using a distribution-free multiple comparison test based on Friedman's rank sums (Hollander and Wolfe 1973).

Results and Discussion

Mean estimates of alfalfa weevil larval intensity obtained using the Tullgren funnel method differed significantly (alpha = 0.05) from bucket-shake sampling estimates in 7 to 28 percent of the fields, depending on larval instar (Table 1). Of the fields with differing estimates from the two sampling methods, there was a lack of consistency concerning which method gave higher estimates. Generally, however, the Tullgren funnel gave higher mean estimates than the bucket-shake for first and third instar larvae, whereas the bucket-shake method produced higher mean estimates of second and fourth instar larvae.

The plastic bag/Tullgren funnel sampling method generally underestimated population levels of alfalfa weevil larvae when compared to estimates obtained from bucket-shake sampling. This underestimation was reasonably consistent, however, and calibration equations are presented (Table 2) to convert Tullgren funnel estimates to bucket-shake estimates. Regression coefficients of determination ($R^{2)}$ (Table 2) indicate that a simple linear regression model can be used to convert mean estimates obtained from the Tullgren funnel method to bucket-shake sampling estimates for all stages except for first instar.

Regression analysis (Table 2) indicated that sweep-net sampling could be used to predict stem intensities of fourth instar larvae (P = 0.001, R^2 = 0.75); however prediction of mean estimates of other larval stages was very poor. Although the sweep-net gave poor estimates of weevil intensities (as estimated by the bucket-shake method), the sweep-net was more precise (as indicated by lower average coefficients of variation) than either the bucket-shake or the Tullgren funnel method for estimating third and fourth instar larval abundance (Table 3). This is most likely due to the contagious distribution of alfalfa weevil larvae among stems at the fairly low population levels encountered in this study. Since the sweep-net is a volumetric sampling method, sweeping literally thousands of individual alfalfa stems per sample, inter-stem variability in larval intensity would not be an important source of variability in sample results. Although sweep-net sampling can be used to give fairly reliable estimates of fourth instar larval intensity per alfalfa stem, sweep-net sampling is not recommended for estimation of abundance of other larval instars and total number of larvae. A number of investigators continue to use sweep-net sampling in order to compare results with data gathered in previous years, however, results of this

Table 1. Comparison of mean estimates of alfalfa weevil larvae intensity per stem obtained from bucket-shake and plastic bag/Tullgren funnel sampling.

	Percent fields with significantly greater means*		Average difference in mean larvae per stem†	
Instar	Tullgren	Bucket-shake	Tullgren	Bucket-shake
First	14	0	0.08	–
Second	7	21	0.06	0.11
Third	7	0	0.14	–
Fourth	7	21	0.25	0.35

* α = .05, using Student's T-test. Sampling was conducted in 14 fields.
† Calculated only for those fields in which the mean estimates from the two sampling methods were significantly different (see footnote* above).

Table 2. Regression parameters for predicting mean number of alfalfa weevil larvae per alfalfa stem (obtained from bucket-shake sampling) from mean estimates of larvae per stem obtained from plastic bag/Tullgren funnel and sweep-net sampling.

Sample method	Instar	*P*-values of regression model	R^2	Regression slope coefficient* b_1
Tullgren funnel	1st	0.261	0.10	NS
	2nd	0.001	0.59	1.45
	3rd	0.001	0.83	1.10
	4th	0.001	0.62	1.33
Sweep-net	1st	0.042	0.32	0.45
	2nd	0.115	0.21	NS
	3rd	0.141	0.19	NS
	4th	0.001	0.75	0.07

* Model: $y = b_0 + b_1X$, where y = mean number of larvae per stem estimated from Bucket-shake sampling, X = mean number of larvae per stem from plastic bag/Tullgren ***or*** mean number per sweep, b_0 = the y intercept, and b_1 = the slope. Regression coefficients are listed only for significant regressions (α = .05). No intercept values (b_0) were significantly different from zero. Fourteen pairs of observations were used in the regression analysis.

Table 3. Mean coefficients of variation for three sampling methods used to estimate alfalfa weevil larval abundance.

	Sample Method			
Larval instar	Sweep-net	Bucket-shake	Tullgren funnel	n*
First	168	203	134	7
Second	64	98	97	10
Third	43 a†	81 b	72 b	12
Fourth	39 a	81 b	72 b	11

* Number of sets of matched observations used for multiple comparison tests.
† Means followed by different letters within a row are significantly different at $\alpha = .05$ (Friedman's Rank Sums).

experiment support the conclusions of Blickenstaff and Huggans (1969) and Cothran and Summers (1972) that sweep-net sampling is inappropriate for estimating abundance of alfalfa weevil larvae.

Although cost, particularly in terms of labor requirements, is an important consideration in sampling method evaluation, detailed cost data were not taken in this study. Generally the Tullgren funnel method took considerably longer than the other methods because of additional handling time in transporting the samples to the Tullgren funnel facility and in loading and unloading the funnels. Total number of samples taken per day using this method is limited by the number of available Tullgren funnels. Field time required for bucket-shake and sweep-net sampling were comparable, however more time was needed to sort and count the larger number of larvae collected from sweep-net sampling.

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