

Sequential sampling plans for estimating European corn borer (Lepidoptera: Crambidae) and corn earworm (Lepidoptera: Noctuidae) larval density in sweet corn ears

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Abstract

We developed a flexible fixed-precision sequential sampling plan for estimating the density of European corn borer, *Ostrinia nubilalis* Hübner and corn earworm, *Helicoverpa zea* (Boddie), larvae, using infestation data collected from 1994 to 2000. The purpose of each sampling plan was to provide statistically sound estimates of larval densities for each pest in sweet corn ears, near harvest, with minimal cost. Sweet corn variety plots and commercial production fields were sampled to obtain a wide range of *O. nubilalis* and *H. zea* densities typically found in sweet corn, in the Midwestern USA. Sampling parameters were estimated from 84 and 68 data sets for *O. nubilalis* and *H. zea*, respectively. An additional 15 independent data sets, for each species, were used to validate the fixed-precision sequential sampling plans with resampling software. Dispersion patterns for *O. nubilalis* and *H. zea* were determined to be random and uniform, respectively, from Taylor's power law. For *O. nubilalis*, at densities of 0.24–4.08 larvae/ear, an average sample number (ASN) of only 38 ears was necessary to achieve a desired precision level (SE/mean) of 0.25. As the precision level was increased to 0.10, average sample size increased to 227 ears. For *H. zea*, at densities of 0.20–2.05 larvae/ear, an ASN of 27 ears was required to achieve a desired precision level of 0.25. As the precision level was increased to 0.10, sample size increased to 160 ears. The sequential sampling plans will be useful to researchers for quantitative assessment of integrated pest management (IPM) strategies, via rapid estimation of larval density per ear, the primary determinant of IPM efficacy and product quality near harvest. Additionally, these plans can be used to determine the background density necessary for estimating the frequency of *O. nubilalis* or *H. zea* larvae found in transgenic Bt sweet corn ears expressing *Bacillus thuringiensis* proteins.

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1. Introduction

European corn borer, *Ostrinia nubilalis* Hübner, and corn earworm, *Helicoverpa zea* (Boddie), are the two major lepidopteran pests of sweet corn, *Zea mays* (L.), in the north central United States (Bartels and Hutchison, 1995; Flood et al., 1995). A sampling program for estimating *O. nubilalis* egg mass or early instar infestations in sweet corn has been proposed to facilitate timely use of insecticides (e.g., Shelton et al., 1986). However, there are currently no sequential sampling plans available for estimating the density of *O. nubilalis* or *H. zea* larvae in ears near harvest. The need for precision-based density estimation recently became more critical with the commercial development

of sweet corn hybrids genetically engineered to express various proteins of the soil-borne bacterium, *Bacillus thuringiensis* (Lynch et al., 1999; Burkness et al., 2002). In an effort to minimize the evolution of insect resistance to Bt corn, the primary resistance management tactic has been the use of non-Bt corn refugia planted near Bt corn fields (Ostlie et al., 1997). One method for monitoring Bt resistance in *O. nubilalis* and *H. zea* is the “in-field screen” which requires larval density to be determined from nearby non-Bt fields for the target species, *O. nubilalis* or *H. zea* (Venette et al., 2000a, b, 2002). The plans we present here will allow researchers to estimate larval density from non-Bt sweet corn with a high degree of precision (e.g., 0.10) which is desired for research applications (Burkness et al., 1998; O'Rourke et al., 1998). In addition, these plans should be useful for rapid assessment of the efficacy of new control tactics in sweet corn as larval infestation in the

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ear near harvest is a primary determinant of the final product marketability and efficacy of an integrated pest management (IPM) program (e.g., Burkness et al., 2002). The focus of this paper is to develop and validate fixed-precision sequential sampling plans (Green, 1970) to estimate the larval density of *O. nubilalis* and *H. zea* in near-harvest sweet corn ears.

2. Materials and methods

2.1. General

Sweet corn fields were sampled from 1994 to 2000 at University of Minnesota Agricultural Research and Outreach Centers located at all major corn growing regions in the state, including Rosemount, Waseca, Lamberton, Morris, Crookston and Becker, MN. Commercial sweet corn fields were also sampled near Owatonna, MN. The primary ears of one of four commercially available processing varieties, 'Bonus', 'Empire', 'Heritage' (Syngenta Corp., formerly Novartis, Nampa, ID) and 'Jubilee' (Northrup King, Golden Valley, MN) were examined for the presence of larvae for each sample date. Experimental plots located at the Research and Outreach Centers were either 4 or 8 rows wide and 9 m long or 20 rows wide and 15 m long (Burkness et al., 2001, 2002). Commercial production fields ranged from 0.01 to 100 ha. In all studies and commercial fields, row spacing was 76 cm.

At harvest, when primary ears approached $\approx 70\%$ moisture (ca. 90 d after planting), ears were hand-harvested from 40 to 200 plants, depending on plot size. For experimental plots, 10–20 consecutive ears were arbitrarily selected from multiple interior rows of plots with plants on the ends of each row being skipped. For larger commercial fields, 10–20 consecutive ears were arbitrarily selected from multiple locations within each field to provide greater coverage. Ears were typically harvested and evaluated by 4–6 people per location. Ears were placed into burlap sacks and taken to the field edge for immediate evaluation. Husks were removed and ears were examined for the presence of lepidopteran larvae; feeding damage was also noted (e.g., Bartels and Hutchison, 1995). All instars for both *O. nubilalis* and *H. zea* were included in the analysis as these plans are designed to estimate total larval density. Total larval density is also used in the existing in-field resistance monitoring protocol described by Venette et al. (2000a). A total of 182 samples were collected during the study, including 99 for *O. nubilalis* and 83 for *H. zea*.

2.2. Data analysis

Taylor's power law (Taylor, 1961) was used to summarize the mean–variance relationship for both

species within the context of Green's fixed-precision sampling plan (Green, 1970; Naranjo and Hutchison, 1997). Taylor's power law parameters, a and b , were estimated from all data sets not used for validation, including 84 and 68 data sets for *O. nubilalis* and *H. zea*, respectively. Taylor's a parameter was determined by taking $\text{antilog}[a]$ from regression analysis and Taylor's b parameter was taken directly from regression analysis. Sequential sampling plans were developed for fixed-precision levels (D) of 0.10 and 0.25 for larvae of *O. nubilalis* and *H. zea* where $T_n \geq (an^{1-b}/D^2)^{1/(2-b)}$ (Green, 1970). A precision level of 0.25 is generally accepted for pest sampling in IPM programs (Southwood, 1978), while a precision level of 0.10 is generally desired for research purposes. Using Green's method, the resampling for validation of sampling plans (RVSP) program was used to validate the sequential sampling plans for each species (Naranjo and Hutchison, 1997). RVSP

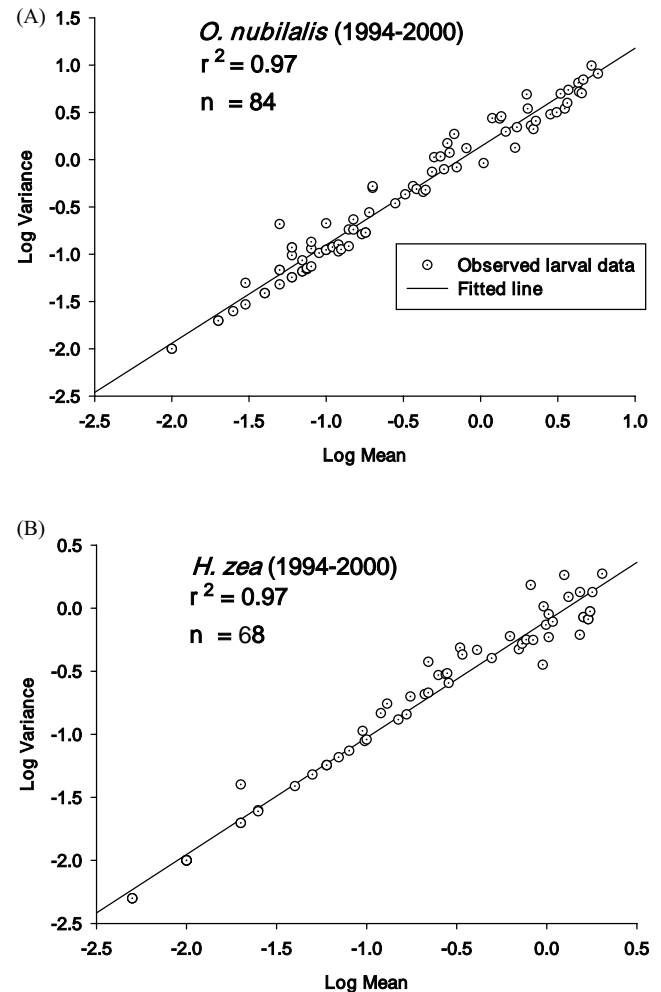


Fig. 1. (A) Taylor's power law regression for *O. nubilalis*, 1994–2000, where $a = 1.376$, $b = 1.039$, $\text{SE}(b) = 0.0215$, for mean density range of 0.01–5.76 larvae/ear. (B) Taylor's power law regression for *H. zea*, 1994–2000, where $a = 0.794$, $b = 0.926$, $\text{SE}(b) = 0.0185$, for mean density range of 0.005–2.03 larvae/ear.

Table 1
Resampling results for validation of Green's fixed-precision sequential sampling plan for *O. nubilalis* with pre-set precision (D)=0.25^a

Data set	Observed density ^b	Average statistics for 500 sequential sampling simulations						
		Density	Precision (D)			Sample size		
			\bar{X}	\bar{X}	Max.	Min.	\bar{X}	Max.
Desired $D=0.25$								
1	0.24	0.24	0.26	0.34	0.20	94	178	39
2	0.31	0.32	0.23	0.31	0.17	71	121	37
3	0.32	0.35	0.27	0.34	0.19	66	138	30
4	0.39	0.41	0.24	0.39	0.16	57	107	23
5	0.50	0.56	0.31	0.42	0.22	45	102	15
6	0.56	0.61	0.28	0.40	0.18	41	100	14
7	0.59	0.63	0.26	0.40	0.17	39	76	15
8	0.61	0.65	0.26	0.36	0.16	37	71	11
9	0.70	0.71	0.22	0.27	0.14	33	59	19
10	0.76	0.83	0.27	0.39	0.17	30	61	10
11	1.31	1.36	0.21	0.32	0.10	18	29	10
12	1.90	1.98	0.20	0.35	0.09	13	21	10
13	2.24	2.29	0.21	0.35	0.10	11	21	10
14	2.52	2.52	0.23	0.37	0.12	11	18	10
15	4.05	4.03	0.17	0.32	0.04	10	11	10
Overall mean			0.24			38		

^a $T_n \geq (an^{(1-b)} / D^2)^{1/(2-b)}$, where T_n =cumulative number of individuals sampled, $a = 1.376$, $b = 1.039$, $D = 0.25$, n =number of samples (Green, 1970).

^b Observed mean density values taken from field-collected independent validation sets (mean number of larvae/ear), not used to develop the sampling plan.

requires the use of independent data sets for validation. Thus, 15 data sets representing a range of low, medium, and high densities were selected at random from both the 99 *O. nubilalis* data sets and the 83 *H. zea* data sets to serve as validation data sets (Naranjo and Hutchison, 1997). The validation data sets reflected the range of larval densities observed among sampled sweet corn fields for each species. The RVSP software was used to resample each of 15 data sets until the stop line had been reached (Naranjo and Hutchison, 1997). In addition to the initial fixed-precision levels of 0.25 and 0.10, a minimum sample size of 10 ears was used for all simulations. Resampling was repeated 500 times for each data set, producing the average, minimum and maximum precision level and the average, minimum and maximum sample size.

3. Results and discussion

3.1. *O. nubilalis*

Mean larval densities for the 99 *O. nubilalis* data sets ranged from 0.01 to 5.76 larvae/ear. Taylor's power law (Taylor, 1961) parameters were calculated from 84 data sets; 15 remaining data sets, with a density range from

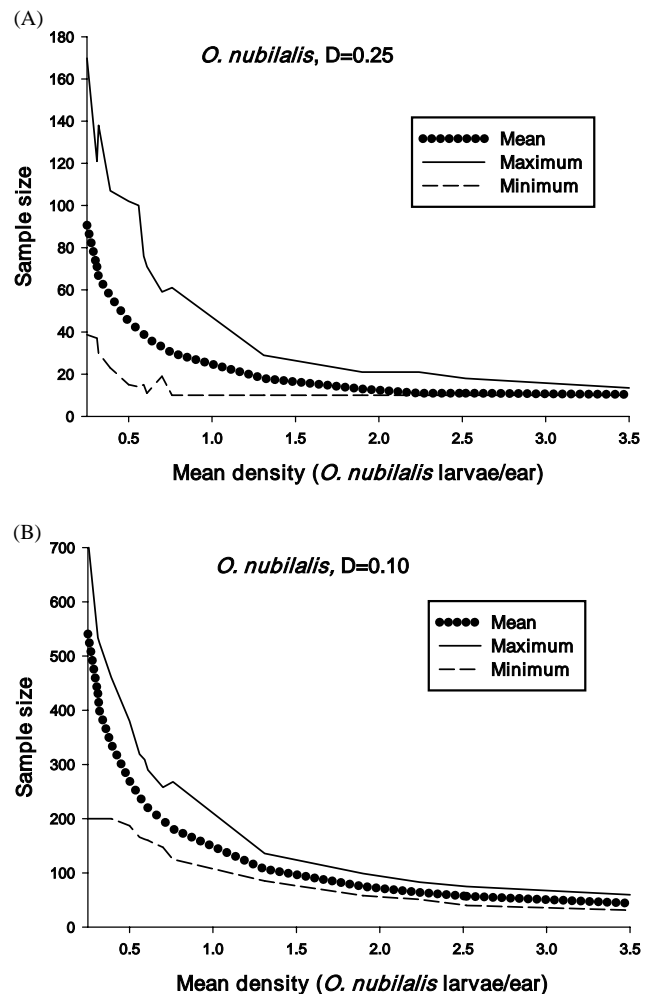


Fig. 2. Summary of resampling validation analysis showing actual ASN for Green's sequential sampling plan over a range of *O. nubilalis* densities. Green's parameters: (A) $D = 0.25$, $a = 1.376$, $b = 1.039$; (B) $D = 0.10$, $a = 1.376$, $b = 1.039$.

Table 2
Resampling results for validation of Green's fixed-precision sequential sampling plan for *O. nubilalis* with pre-set precision (D)=0.10^a

Data set	Observed density ^b	Average statistics for 500 sequential sampling simulations						
		Density	Precision (D)			Sample size		
		\bar{X}	\bar{X}	Max.	Min.	\bar{X}	Max.	Min.
Desired $D=0.10$								
1	0.24	0.24	0.11	0.12	0.10	559	747	200
2	0.31	0.31	0.09	0.10	0.08	431	534	200
3	0.32	0.34	0.11	0.12	0.10	399	524	200
4	0.39	0.40	0.10	0.13	0.08	338	461	200
5	0.50	0.51	0.13	0.16	0.11	270	380	187
6	0.56	0.57	0.11	0.14	0.10	241	319	166
7	0.59	0.61	0.11	0.13	0.09	227	309	162
8	0.61	0.63	0.11	0.13	0.09	220	290	160
9	0.70	0.70	0.09	0.10	0.07	197	258	147
10	0.76	0.77	0.11	0.13	0.09	181	268	125
11	1.31	1.31	0.09	0.10	0.07	107	136	85
12	1.90	1.92	0.09	0.11	0.06	75	99	58
13	2.24	2.26	0.09	0.12	0.06	64	83	51
14	2.52	2.56	0.10	0.13	0.08	57	75	40
15	4.05	4.08	0.09	0.13	0.06	37	51	26
Overall mean			0.10			227		

^a $T_n \geq (an^{(1-b)}/D^2)^{1/(2-b)}$, where T_n =cumulative number of individuals sampled, $a = 1.376$, $b = 1.039$, $D = 0.10$, n =number of samples (Green, 1970).

^b Observed mean density values taken from field-collected independent validation sets (mean number of larvae/ear), not used to develop the sampling plan.

Table 3
Resampling results for validation of Green's fixed-precision sequential sampling plan for *H. zea* with pre-set precision (D)=0.29^a

Data set	Observed density ^b	Average statistics for 500 sequential sampling simulations						
		Density	Precision (D)			Sample size		
		\bar{X}	\bar{X}	Max.	Min.	\bar{X}	Max.	Min.
Desired $D=0.25$								
1	0.20	0.22	0.28	0.33	0.22	55	139	19
2	0.22	0.25	0.32	0.43	0.25	50	113	15
3	0.25	0.28	0.28	0.36	0.22	43	93	14
4	0.36	0.40	0.26	0.32	0.16	29	55	10
5	0.57	0.62	0.22	0.31	0.00	17	37	10
6	0.59	0.62	0.27	0.47	0.13	18	34	10
7	0.60	0.65	0.29	0.37	0.16	17	39	10
8	0.73	0.79	0.31	0.52	0.15	15	33	10
9	0.88	0.90	0.25	0.40	0.00	12	24	10
10	1.08	1.10	0.29	0.41	0.09	11	21	10
11	1.22	1.22	0.17	0.36	0.00	10	15	10
12	1.22	1.23	0.21	0.45	0.00	10	15	10
13	1.53	1.54	0.24	0.44	0.09	10	15	10
14	1.55	1.51	0.23	0.46	0.00	10	15	10
15	2.05	2.06	0.17	0.32	0.05	10	10	10
Overall mean			0.25			21		

^a $T_n \geq (an^{(1-b)}/D^2)^{1/(2-b)}$, where T_n =cumulative number of individuals sampled, $a = 0.794$, $b = 0.926$, $D = 0.29$, n =number of samples (Green, 1970).

^b Observed mean density values taken from field-collected independent validation sets (mean number of larvae/ear), not used to develop the sampling plan.

0.24 to 4.05 larvae/ear, were used for the resampling validation analyses. Taylor's a and b values were determined to be 1.376 and 1.039, respectively. Taylor's

b value was not significantly different from 1.00 ($P > 0.05$, $t = 1.81$, $df = 83$), indicating a random dispersion pattern for the late instar larvae (Southwood,

1978) (Fig. 1A). Although early instar *O. nubilalis* larvae are often aggregated (e.g., $b > 1$) (Shelton et al., 1986), in part due to their hatching pattern from egg masses (ca. 25 eggs/egg mass), we observed a more random pattern ($b = 1$) for surviving late instar larvae. This is not surprising due in part to the outward movement of the larvae away from the egg mass (Shelton et al., 1986).

Resampling analysis for *O. nubilalis* with precision (D) set at 0.25 resulted in an average sample number (ASN) of 38 ears, ranging from 10 to 94 ears (Table 1). For all data sets combined, over the entire density range, the average precision level ($D = 0.24$) was very close to the desired level of 0.25.

ASN decreased rapidly as mean density increased (Table 1, Fig. 2A). With the exception of the ultra-low densities (e.g., < 0.5 /ear), our analysis suggests that 38–40 ears would generally be adequate for maintaining precision at 0.25. However, the sequential sampling plan can be used to account for any specific *O. nubilalis* density encountered in sweet corn ears at harvest. For IPM purposes, where a crop consultant or processing company representative is monitoring ears for quality control near harvest, the sequential sampling plan with $D = 0.25$ will be useful for minimizing sample size requirements and cost. For research purposes, with the desired precision set at 0.10, higher sample sizes were required (Table 2). On average, to achieve a desired precision level of 0.10, 227 ears must be sampled over the density range represented by the validation data sets (0.24–4.05 larvae/ear) (Table 2, Fig. 2B).

3.2. *H. zea*

Mean larval densities for the 83 *H. zea* data sets ranged from 0.005 to 2.05 larvae/ear. Taylor's power law parameters were calculated from 68 data sets, while the remaining 15 data sets were used for resampling validation analysis. Taylor's a and b values were 0.794 and 0.926, respectively. Unlike *O. nubilalis*, the b value was significantly less than 1.00 ($P < 0.05$, $t = 3.97$, $df = 67$) indicating a uniform dispersion pattern (Southwood, 1978) (Fig. 1B). Given previous results with surviving late instar *H. zea* in sweet corn ears (e.g., Burkness et al., 2002), and their tendency to be cannibalistic (Metcalf and Metcalf, 1993), it is not surprising to observe a uniform dispersion pattern and slightly lower densities of this pest (i.e., maximum of 2.05/ear) compared with *O. nubilalis*.

With the initial desired precision level set at 0.25, the overall actual average $D = 0.22$ (ASN = 27) indicated that a $D = 0.25$ was higher than necessary to achieve the desired precision level (data not shown). This has been observed for other arthropods and illustrates the need for a validation process (e.g., Naranjo and Hutchison, 1997). We therefore relaxed the desired precision level to 0.29 which resulted in an overall actual average $D =$

0.25. The ASN was subsequently reduced to 21 ears (Table 3), ranging from the minimum of 10 ears for high-density fields to a maximum of 139 for low-density fields. As with *O. nubilalis*, the ASN for *H. zea* decreased rapidly as the mean density increased (Table 3, Fig. 3A). For IPM purposes and quality control sampling by processors ($D = 0.25$), and for larval densities exceeding 0.5 larvae/ear, sample sizes should be reasonable, ranging from 10 to 40 ears (Table 3, Fig. 3A). To achieve a desired precision level of 0.10 for research applications, an average of 160 ears must be sampled over the density range represented by the validation data sets (Table 4, Fig. 3B).

Similar density-based, fixed-precision sequential sampling plans have been developed and validated using the resampling approach (Naranjo and Hutchison, 1997) for several insect species, including: aster leafhopper, *Macrosteles quadrilineatus* (Forbes) (O'Rourke et al., 1998), rusty grain beetle, *Cryptolestes ferrugineus*

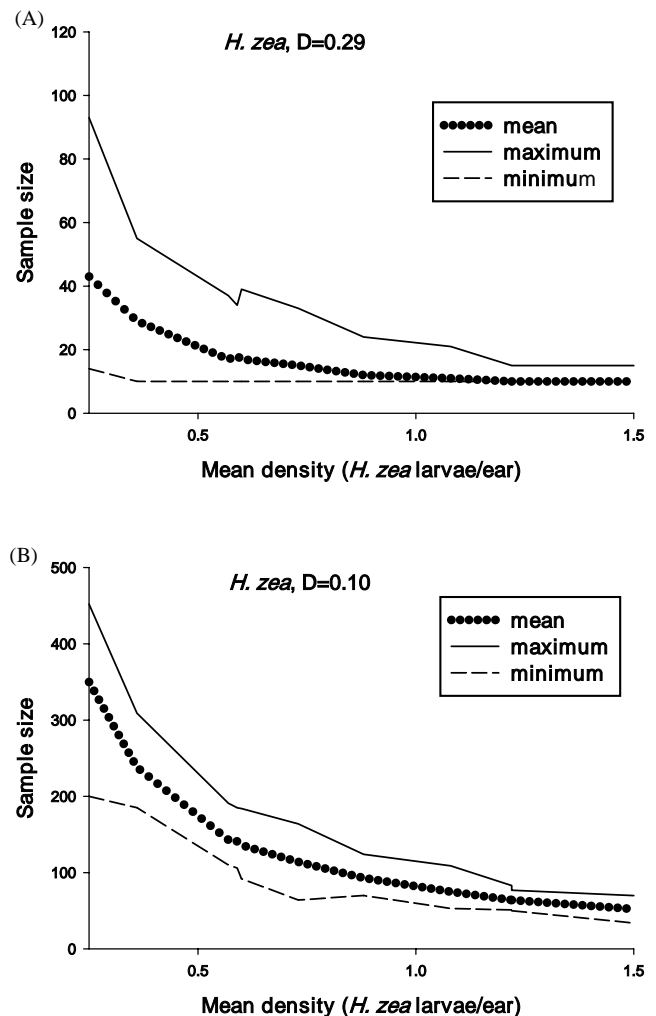


Fig. 3. Summary of resampling validation analysis showing actual ASN for Green's sequential sampling plan over a range of *H. zea* densities. Green's parameters: (A) $D = 0.29$, $a = 0.794$, $b = 0.926$; (B) $D = 0.10$, $a = 0.794$, $b = 0.926$.

Table 4

Resampling results for validation of Green's fixed-precision sequential sampling plan for *H. zea* with pre-set precision (D)=0.10^a

Data set	Observed density ^b	Average statistics for 500 sequential sampling simulations						
		Density	Precision (D)			Sample size		
		\bar{X}	\bar{X}	Max.	Min.	\bar{X}	Max.	Min.
Desired $D=0.10$								
1	0.20	0.20	0.10	0.11	0.09	446	614	200
2	0.22	0.23	0.11	0.13	0.10	397	559	200
3	0.25	0.26	0.10	0.11	0.09	350	452	200
4	0.36	0.37	0.09	0.10	0.08	238	309	185
5	0.57	0.58	0.08	0.09	0.07	143	191	110
6	0.59	0.60	0.10	0.12	0.08	141	185	106
7	0.60	0.62	0.12	0.12	0.09	136	184	92
8	0.73	0.73	0.11	0.13	0.09	114	164	64
9	0.88	0.88	0.09	0.11	0.07	93	124	70
10	1.08	1.09	0.11	0.13	0.09	75	109	53
11	1.22	1.24	0.09	0.12	0.06	64	83	51
12	1.22	1.24	0.07	0.11	0.05	64	77	50
13	1.53	1.55	0.11	0.14	0.07	51	69	32
14	1.55	1.55	0.11	0.14	0.07	51	71	36
15	2.05	2.07	0.09	0.12	0.06	37	51	28
Overall mean			0.10			160		

^a $T_n \geq (an^{(1-b)}/D^2)^{1/(2-b)}$, where T_n =cumulative number of individuals samples $a = 0.794$, $b = 0.926$, $D = 0.10$, n =number of samples (Green, 1970).

^b Observed mean density values taken from field-collected independent validation sets (mean number of larvae/ear), not used to develop the sampling plan.

(Stephens), (Subramanyam et al., 1997), striped cucumber beetle, *Acalymma vittatum* (L.), (Burkness and Hutchison, 1998), and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), (Hamilton et al., 1998). This approach illustrates that when adequate independent data sets are used for validation, the final sequential plans can be used with confidence to ensure that the desired fixed-precision levels are achieved.

The fixed-precision sequential sampling plans for *O. nubilalis* and *H. zea* will be useful for rapid estimation of larval densities in sweet corn for large-scale research programs designed to assess the efficacy of multiple control tactics, including conventional foliar insecticides (e.g., Bartels and Hutchison, 1995; Burkness et al., 2002). Additionally, these plans will be useful for estimating the "background" density of either species when using the in-field resistance monitoring method for transgenic Bt corn (e.g., Venette et al., 2000a, b). Venette et al. (2000a) described a method for determining the initial phenotypic frequencies of surviving larvae on Bt corn, by estimating the ratio of the number of late instars found in Bt corn ears relative to the larval density found in adjacent plantings of non-Bt corn. The sequential sampling plans presented in this paper will enable researchers to determine background densities of either *O. nubilalis* or *H. zea* on non-Bt corn with a known level of precision. For IPM purposes, the sequential plans have the potential to save crop consultants and sweet corn processors significant time

while estimating population densities of either *O. nubilalis* or *H. zea* with minimal cost.

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