

## Control Decision Rule for European Chafer (Coleoptera: Scarabaeidae) Larvae in Field Corn

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**ABSTRACT** After greenhouse and outdoor microplot experiments, a critical density of two third instars per microplot for third instars of European chafer, *Rhizotrogus (Amphimallon) majalis* (Razoumowsky), in corn, *Zea mays* L., was derived. On average, the number of missing or damaged plants increased  $\approx 8\%$  from zero to two larvae per 900 cm<sup>2</sup>. Furthermore, 23 fields in 2 yr were sampled for larvae along transects by using a golf cup cutter as the sampling tool and the critical density of 0.2 larva per sampling unit as the critical density. The sampling unit was one golf cup cutter with a diameter of 10.8 cm or 91.4 cm<sup>2</sup> (10 sampling units  $\approx 900$  cm<sup>2</sup>  $\approx 1$  foot<sup>2</sup>). Fieldwide means and variation were modeled to Taylor's power law,  $a = 1.42$  and  $b = 1.47$ , and 20 of 23 fields fit the negative binomial probability distribution. Wald's formula for a sequential sampling plan was most accurate and least time-consuming, according to the operating characteristic and the average sample number function, relative to Iwao's and converging lines formulae. Percentage of sand, topography, soil bulk density, and proximity to trees were measured as potential predictors of areas with high larval density. Percentage of sand and soil bulk density were significant predictors, and topography and proximity to trees were not significant predictors. Field areas where the percentage of sand is high and the soil bulk density is low to moderate or where the percentage of sand is moderate and the soil bulk density is low should be chosen as sampling locations.

**KEY WORDS** sequential sampling, critical density, European chafer, corn, economic threshold

SINCE 1996, WE HAVE observed that in some areas of southern Ontario and Michigan with sandier soils, field corn, *Zea mays* L., has been frequently and significantly damaged in the spring by European chafer, *Rhizotrogus (Amphimallon) majalis* (Razoumowsky) larvae (unpublished data). The damage has been observed in corn seedlings from emergence until approximately the five-leaf stage. Larvae feed below ground, favoring the hypocotyls and primary root tissue, leaving plants stunted, wilted, or dead from direct damage by chewing or by the entry of secondary rot organisms through wounds. We have observed fields with the plant stand decimated in some of the sandiest areas, such as on the knolls, and in some fields plant loss has exceeded 30% overall. The larvae overwinter as late second to third instars and begin feeding ravenously as soon as the seeds germinate, even when the soil temperature is cool. Corn is documented as a potential or minor host for European chafer (Tashiro et al. 1969), but the recent economic significance of European chafer larvae in corn is unprecedented.

Larvae are controlled with granular insecticides or seed treatments (Hooker and Schaafsma 2004), but currently control decisions are prophylactic and not based on sampling. No forecasting methods are available for the European chafer in field corn, even though it has a single, annual generation; it is nonmigratory; and it overwinters as a mature larva (Tashiro et al. 1969).

Reports on sampling and thresholds for European chafer and other white grubs for field crops are limited. Burrage and Gyrisco (1954a, b) demonstrated that smaller sampling units were more efficient than larger units and that larval distribution in pastures was patchy, fitting the negative binomial distribution (NBD). Ives and Warren (1965) developed sequential sampling plans with a NBD for white grubs, *Phyllophaga*, and *Serica* spp., for use in forestry. Ng et al. (1983a, b) fitted counts of Japanese beetle larvae, *Popillia japonica* Newman, to the NBD by using small, square soil samples on golf course fairways and developed a sequential sampling plan. Nyrop et al. (1995) used small, circular plugs in residential lawns to formulate a double sampling plan for European chafer. They used sitewide characteristics such as lawn age and percentage of shade, to determine whether a site required sampling. Furthermore, within lawns needing sampling, they developed a relationship between sitewide density and patch size, or density within a patch to suggest a threshold.

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In turf, a nominal threshold of 0.25 larva per 11-cm-diameter plug is used (Nyrop et al. 1995), but it does not take into account any economic cost–benefit ratios. A similar threshold or critical density for use as part of a sampling protocol for third instars in corn is not available and is needed.

Large field sizes for corn pose an obstacle for sampling, because larval distributions are likely aggregated as they are in turf and pasture (Burrage and Gyrisco 1954a, b; Nyrop et al. 1995). Because European chafer larvae are controlled at planting with in-furrow or seed treatment insecticides, it is difficult to localize an application within a field. A control decision for an entire field must be made from samples collected from a small proportion of the field. If samples are collected from areas where insect density is low, and potentially below a critical density, an incorrect decision would be made. However, if areas of highest densities are predictable, then the probability of not applying a treatment when one is necessary is reduced.

Distributions of European chafer larvae are often influenced by soil properties (Shorey et al. 1960, Tashiro et al. 1969, Nyrop et al. 1995) and proximity to trees (Tashiro et al. 1967), and these factors might be useful predictors of high larval density. The goal of this study was to develop a control decision rule for European chafer larvae in field corn as the basis of an integrated pest management approach by using the following objectives: to determine a critical density or threshold where larval damage necessitates action; to propose an accurate, efficient, sequential sampling plan; and to construct a risk assessment or prediction model based on landscape factors for use in implementing the sampling plan.

## Materials and Methods

**Critical Density Determination.** Corn was planted in 30 by 30 by 30-cm microplots on 1 May 2003 and 11 November 2003 at Ridgetown College, Ridgetown, Ontario, Canada, in a randomized complete block design with six replications. Each microplot was a galvanized metal cube open at opposite ends, placed in a plastic tray of similar dimensions but 7.5 cm in depth. The tray was partially filled with a mixture (1:1) of moistened heat pasteurized field soil and a standard greenhouse soil with a mixture (1:1:1) of peat, perlite, and vermiculite. Three corn seeds were planted by hand to a depth of 5 cm in each microplot (hybrid Pioneer D73, pretreated with a fungicide MAXIM XL 324 FS (fludioxonil + metalaxyl-M, 2.5 + 0.96 g [AI]) / liter, Syngenta Crop Protection) at a rate of 3.5 g AI/100 kg seed. The trial planted in May was conducted outdoors on a clean gravel base under ambient conditions, and the trial planted in November was conducted in the greenhouse.

Third instars of European chafers were collected from a natural infestation in turf and placed in cold storage at  $\approx 5^{\circ}\text{C}$ . On the day of planting, 0, 1, 2, 4, 8, or 16 larvae were placed on the soil surface in each microplot. Injured or damaged larvae that did not

quickly burrow into the soil were replaced by healthy larvae until all burrowed into the soil. Plant stand and damage were assessed weekly. Wilted plants were considered damaged. Four weeks after planting, on 29 May and 9 December for the outside and greenhouse trials respectively, corn plants in each microplot were cut at the soil level, and the total fresh weight was measured. The soil in each microplot was sifted to recover and count live and dead larvae.

Analysis of variance (ANOVA) was performed separately for data from the spring and fall trials. Data were tested for homogeneity (residual errors plotted against predicted errors), and a normal distribution (Shapiro–Wilks test), to determine whether a transformation was necessary. Lund's table was used to determine the presence of outliers. Orthogonal contrasts were made between treatments containing zero, one, and two larvae. All statistical computations were performed using SAS version 8.2 (SAS Institute 2004). The type I error rate ( $\alpha$ ) was set at 0.05 for all statistical tests.

**Field Surveys.** Data were collected from five agricultural fields in southwestern Ontario in spring 2002 (17–29 May), one field in southwestern Ontario in fall 2002 (6 November), 15 fields in southwestern Ontario in spring 2003 (6 May–6 June), and two fields in central Michigan in spring 2003 (1–2 May). These were fields with reported high densities of larvae. Two types of data were collected: sample counts of larvae along transects in the field and measurements of variables describing characteristics of the field where sampling occurred. Sampling and variable measurements were done when third instars were actively feeding (Tashiro et al. 1969).

Counts of third instars were made along field transects and used to calculate densities. Sample units were taken at 50-m intervals along transects with the initial sample unit 25 m from the field edge. Transects were 50 m apart and 25 m or less, depending on the field width, from the field edge. The number of sample units in a field ranged from 18 to 70, depending on field size. Each sample unit consisted of eight soil plugs arranged in a 9 by 3-m rectangle (27 m<sup>2</sup>) with 3 m between samples. Each plug was 10.8 cm in diameter and  $\approx 10$ –15 cm in depth taken with a golf cup cutter (Nyrop et al. 1995). Counts from the eight plugs were averaged where one sampling unit is one soil plug from a golf cup cutter with an area of 91.4 cm<sup>2</sup> (10 sampling units  $\approx 900$  cm<sup>2</sup>  $\approx 1$  foot<sup>2</sup>).

**Risk Assessment Model.** The following field characteristics were measured: relative elevation (topography), soil texture, soil organic matter, soil bulk density, and proximity of sample units to trees or shrubs. A surveyor's transit level was used to record the elevations from a central point in the field at all sample unit locations along each transect. Relative elevation was calculated by assigning the lowest spot in the field a value of zero.

For three fields in 2002 and all fields in 2003, a 500-g aliquot of the sampled topsoil was taken from each sample unit location, placed in a plastic bag, stored at  $\approx 5^{\circ}\text{C}$  to maintain moisture level, and analyzed for

texture (Sheldrick and Wang 1993) and organic matter (Anonymous 2003). In 2003, bulk density was measured by inserting aluminum cylinders ( $r = 2.37$  cm and  $h = 5.00$  or  $5.15$  cm) with beveled edges  $\approx 2.5$ – $7.5$  cm into the ground. The cylinders were carefully removed using a trowel, the soil was leveled off on top and bottom with a putty knife, and then it was pushed by hand into a plastic bag. The soil was massed, and bulk density was expressed as grams per cubic centimeter. Fields were then classified into three groups of equal number according to bulk density (i.e., fields with lowest and middle and highest bulk densities). Organic matter content was not determined in 2003.

Proximity to trees or shrubs was determined as the distance (meters) from the nearest forest–field border, solitary tree, or hedge–shrub row to the sample unit location. Differences in tree or shrub type, species, height, or canopy cover were not recorded. To maximize the number of data points, especially in small fields, transects were often  $<25$  m from tree or hedgerow.

Field characteristic data were used in a forward stepping analysis of variance (ANOVA) (PROC REG, SAS Institute 2004) to determine whether a relationship existed between some or all of these variables and larval density of the sample unit. Variables and their squared values were added or deleted from the model based on a critical  $F$  value with  $P < 0.20$  (Vyn and Hooker 2002). Variables remaining in the model were reentered with first-level interactions to test for significance. The variance inflation factor was used to detect independence of variables and the  $C_p$  (Mallows) statistic to determine the best prediction equation (Bowley 1999). A risk assessment system was constructed using the significant and independent variables to identify field areas where larval density was greatest. Percentage of sand was used to equally divide the data from all 23 fields into thirds: the low, moderate, and high sand areas. Each of these areas was equally subdivided into areas of lowest, moderate, and greatest bulk density. The means of the nine risk assessment classes were calculated and ordered from highest to lowest such that areas in the field with the lowest larvae density were categorized as risk 9.

**Sequential Sampling Plan Development.** The mean and variance of larval counts were calculated for each field sampled during 2002 and 2003. Natural logarithms of means ( $M$ ) and variances ( $V$ ) were calculated to linearize the data, and the sample variance was regressed on the sample mean to determine the parameters for Taylor's power law (TPL) (Taylor 1961):

$$\sigma^2 = a\mu^b \quad [1]$$

$$\ln(V) = \ln a + b \ln(M) \quad [2]$$

where  $a$  is the inverse natural logarithm of the intercept, and  $b$  is the slope. The sample mean approximates the population mean,  $M \cong \mu$ , and the sample variance approximates the populations variance  $V \cong \sigma^2$ .

Count frequencies, or histograms, for each field were fit to either a Poisson or NBD probability dis-

tribution. If a field fit both or neither distribution type, the field was recorded as such. A chi-square test ( $\alpha = 0.05$ ) was used in SAS PROC FREQ to determine goodness-of-fit. The expected Poisson distribution frequencies were calculated for each field using the following formula (Binns et al. 2000):

$$P(x|\mu) = e^{-\mu} (\mu^x/x!) \quad [3]$$

$$\text{for } x = 0, 1, \dots$$

where  $P(x|\mu)$  should be understood as the probability of getting  $x$  number of individuals given the parameter,  $\mu$ . The expected NBD frequencies were calculated for each field using the following formula (Binns et al. 2000):

$$P(0|\mu,k) = (k/\mu + k)^k \quad [4]$$

$$P(x + 1|\mu,k) = P(x|\mu,k) k + x/x + 1 * \mu/\mu + k \quad [5]$$

$$\text{for } x = 0, 1, \dots$$

The aggregation parameter,  $k$ , of the NBD was modeled as a function of the field mean,  $\mu$ , by using TPL (Binns et al. 2000):

$$k = \mu^2/a\mu^b - \mu \quad [6]$$

A separate  $k$  was calculated for each field. A common  $k$ ,  $k_c$ , was calculated across all fields according to the weighted analysis method of (Bliss and Owen 1958). Precision estimates for  $k_c$ : significance of the regression for  $1/k$  and field means, significance of the intercept, and homogeneity of the  $k$  values between fields, were calculated and evaluated.

Iwao's procedure based on classification intervals was used to calculate stop boundaries (Binns et al. 2000):

$$L_n = n (cd - z_{\alpha/2} \text{sqrt } V/n) \quad [7]$$

$$U_n = n (cd + z_{\alpha/2} \text{sqrt } V/n) \quad [8]$$

$$\text{for } n = 1, 2, \dots (\text{maxn} - 1) \text{ sample units,} \\ \text{and } L_{\text{maxn}} = U_{\text{maxn}} = \text{maxn} \times cd.$$

where  $L_n$  is lower stop boundary,  $U_n$  is upper stop boundary,  $cd$  is critical density, and  $V$  is the variance estimate modeled by TPL when the  $cd$  or threshold is equal to the population mean. The term  $z_{\alpha/2}$  is not an error function but simply defines the distance between the upper and lower boundaries (Binns et al. 2000). With increasing  $n$ , the stop boundaries diverged. A minimum number of sample units,  $\text{minn}$ , was chosen to ensure a representative sample was taken. A maximum number of sample units was chosen,  $\text{maxn}$ , at a point where additional sampling would not provide a significant improvement in classification. A decision is made at  $\text{maxn}$  if the count of larvae was  $</>$   $\text{maxn} \times cd$ . A converging lines classification interval was used to calculate stop boundaries (Binns et al. 2000):

$$L_{\text{minn}} = \text{minn} (cd - z_{\alpha/L} \text{sqrt } V/\text{minn}) \quad [9]$$

$$U_{\text{minn}} = \text{minn} (cd - z_{\alpha/U} \text{sqrt } V/\text{minn}) \quad [10]$$

$$L_{\text{maxn}} = U_{\text{maxn}} = \text{maxn} \times cd. \quad [11]$$

where  $L_{\min}$  is lower stop point at minn,  $U_{\min}$  is upper stop point at minn, and  $L_{\max} = U_{\max}$  is the stop point at maxn. Boundaries are established by joining  $L_{\max} = U_{\max}$  to  $L_{\min}$  and  $U_{\min}$  with two straight lines. The  $cd$ ,  $\alpha$ , and  $V$  are the same as Iwao's procedure. Wald's sequential probability ratio test (SPRT) was used to calculate stop boundaries based on the NBD (Binns et al. 2000):

$$\text{Low intercept} = \ln(\beta/1 - \alpha) / \ln[\mu_1(\mu_0 + k) / \mu_0(\mu_1 + k)] \quad [12]$$

$$\text{High intercept} = \ln(1 - \beta/\alpha) / \ln[\mu_1(\mu_0 + k) / \mu_0(\mu_1 + k)] \quad [13]$$

$$\text{Slope} = k \ln(\mu_1 + k / \mu_0 + k) / \ln[\mu_1(\mu_0 + k) / \mu_0(\mu_1 + k)] \quad [14]$$

where  $\alpha$  and  $\beta$  are the probabilities of miscalculation,  $\mu_0$  and  $\mu_1$  are a range of means with the midpoint equal to  $cd$ , and  $k$  is the aggregation parameter of the NBD and estimated at  $cd$  using TPL.

For all three procedures, the  $maxn$  was determined using the coefficient of variation (CV) as a standard and a prior estimate of the variance ( $V$ ) from TPL at  $\mu = cd$  (Binns et al. 2000):

$$n = V / (\mu * CV)^2 \quad [15]$$

A  $minn$  was not determined but was a variable parameter in the determination and evaluation of each sampling plan. The range tested was from two to four minimum samples.

The operating characteristic (OC) and the average sample number (ASN) functions were calculated for all three classification procedures using the simulation method described by Binns et al. (2000). MathCAD software was used at 1,000 replications per population mean. Random variables were taken from the probability distribution (Poisson or NBD) fitting the majority of the sampled fields. Results were plotted and used to evaluate the effectiveness of the sequential sampling plan. The variables,  $minn$ ,  $\beta$ ,  $\alpha$ ,  $\mu_0$ ,  $\mu_1$ , and  $k$ , were altered within reason for each formula until a steep OC curve with a low ASN curve was achieved. The best OC and ASN curves between formulae were compared by visual inspection according to the same criteria and a final sampling plan was chosen.

**Results**

**Critical Density Determination.** The raw data were normally distributed about the mean. As the density of third instars increased in the microplots, mean fresh weight decreased in the outdoor ( $P < 0.0001$ ) and greenhouse ( $P = 0.0005$ ) trials (Fig. 1a, b). Although partitioning of the regression showed a significant linear and quadratic relationship for the outdoor data between zero and eight larvae per microplot, only the linear relationship was used. A quadratic equation implies an increase in fresh weight beyond 16 larvae per microplot; such a situation is not intuitively logical (Higley and Pedigo 1996). The regression partition

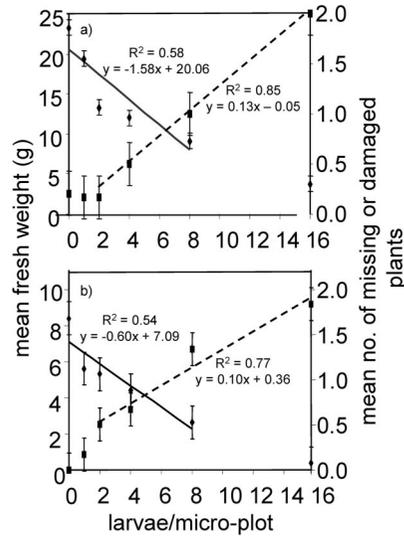


Fig. 1. Relationship between increasing European chafers third instar density in (a) outdoor and (b) greenhouse 30 by 30 by 30-cm microplots and mean fresh weight (grams) (diamonds) and number of damaged and missing plants (squares) per three corn plants 28 days after planting. Lines represent best-fit equations for the linear portion of the curve. Fresh weight data at the level of 16 larvae per microplot were not included because of the large and variable number of missing plants. Standard error indicated above and below the means.

was only linearly significant between zero and eight larvae per microplot for the greenhouse trial. The slopes and intercepts of the lines-of-best-fit for both trials were significantly different from zero ( $P < 0.05$  for all).

As the density of third instars increased in the microplots, the mean number of damaged and/or missing plants increased for the outdoor ( $P < 0.0001$ ) and greenhouse ( $P < 0.0001$ ) trials (Fig. 1a, b). Only the linear partition of the regression from two to 16 larvae per microplot was significant for both trials. The slopes of the lines-of-best-fit were significantly different from zero ( $P < 0.05$ ); however, the intercepts were not ( $P > 0.05$ ) for both trials.

Damage means increased with larval density by orthogonal contrasts, and these were used to make a critical density decision. A significant difference in fresh weight was found between zero and one larva per microplot for both outdoor and greenhouse trials (Table 1). A significant difference in the number of missing and/or damaged plants was shown between zero and eight larvae per microplot in the outdoor trial and between zero and four larvae per microplot in the greenhouse trial. A critical density of two larvae per 30 by 30-cm or 0.2 larva per sampling unit was chosen where the sampling unit was one golf cup cutter with a diameter of 10.8 cm or 91.4 cm<sup>2</sup> (ten sampling units  $\approx$  900 cm<sup>2</sup>  $\approx$  1 foot<sup>2</sup>). A minimum of four larvae per microplot resulted in an unacceptable number of missing plants. One larva per microplot reduced fresh

**Table 1. Orthogonal contrasts for zero and successive numbers of European chafer third instars per microplot for fresh weight and damaged and/or missing corn plants planted at Ridgeway College in outdoor and greenhouse environments during 2003**

Contrast level	Fresh wt				Damaged/missing plants			
	F value		P value		F value		P value	
	OD <sup>a</sup>	GH <sup>b</sup>	OD	GH	OD	GH	OD	GH
0-1	7.21	4.85	0.0130	0.0371	0.01	0.37	0.9043	0.5468
0-2	47.61	5.93	0.0001	0.0224	0.01	3.36	0.9043	0.0788
0-4					0.79	5.97	0.3815	0.0220
0-8					5.81	23.85	0.0239	0.0001

<sup>a</sup> Outdoors.  
<sup>b</sup> Greenhouse.

weight at 4 wk. Two larvae, as a point between one and four larvae per microplot, were chosen as the critical density and a best estimate for an economic threshold.

**Sequential Sampling Plan Development.** A significant regression ( $P < 0.0001$ ,  $R^2 = 0.94$ ) of the variance on the mean allowed determination of the  $a$  and  $b$  parameters for TPL (Fig. 2). TPL for third instars in agricultural fields is as follows:

$$\sigma^2 = 1.42 \mu^{1.47} \quad [16]$$

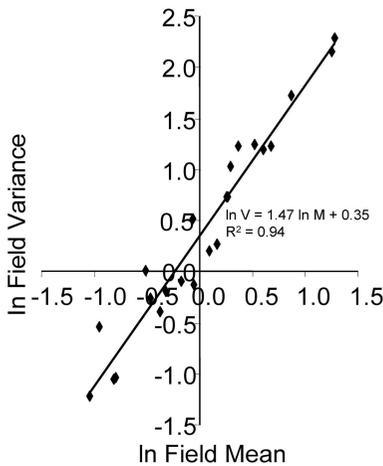
for sample units consisting of eight 11-cm-diameter plugs  $\cong$  eight 1-dm<sup>2</sup> samples.

Histograms were constructed for each field. An example for a field sampled in Charlotte, MI, during 2003 (Fig. 3). The frequency of sample unit counts containing 0, 1, 2, . . . , 10 third instars was represented against the expected NBD or Poisson frequency of sample counts for a given mean and  $k$  value modeled from TPL. A chi-square test was used to detect significant differences from the count frequencies and the expected distribution frequencies (Table 2). If the expected and count frequencies were not significantly different, the count frequency fit the expected fre-

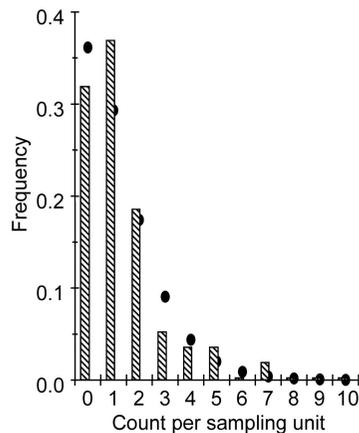
quency distribution. Of the 23 fields sampled, 13 fit both NBD and Poisson distribution, one fit only the Poisson, seven fit only the NBD, and two fit neither. Because counts in 20 of 23 fields fit the NBD, it was selected to generate random variables for simulation and evaluation of a proposed sampling plan.

The  $k_c$  value from the weighted analysis method (Bliss and Owen 1958) was 2.04 for third instars in fields. The precision of  $k_c$  was estimated by the following tests: the individual  $k$  values were homogeneous between fields ( $\chi^2 = 3.16$ ,  $df = 22$ ,  $P > 0.05$ ), there was a nonsignificant relationship between field means and  $1/k$  values ( $R^2 = 0.045$ ,  $n = 23$ ), and the intercept was nonsignificantly different from zero ( $t = 1.15$ ,  $P = 0.264$ ).

The distribution of third instars was over dispersed or aggregated spatially, departing from the line of Poisson expectation ( $V = M$ ) and closely following the line of negative binomial expectation with a common  $k$  ( $V = M + M^2/k_c$ ) and the line corresponding to TPL ( $V = aM^b$ ) (Fig. 4). Chi-square results from Table 1 can be visualized in Fig. 4 where fields (data points) with means  $< 1.0$  larva per sampling unit fol-



**Fig. 2.** Variance-mean relationship for European chafer third instars in 23 agricultural fields during 2002 and 2003. Larvae were counted in 18-70 sample units per field. Each sample unit was eight 11-cm-diameter plugs in a 27-m<sup>2</sup> area in the middle of a 50 by 50-m block. The line-of-best-fit has a slope of 1.47 (SE = 0.08,  $P < 0.0001$ ), intercept of  $\ln 0.35$  (SE = 0.05,  $P < 0.0001$ ), and  $R^2 = 0.94$ .



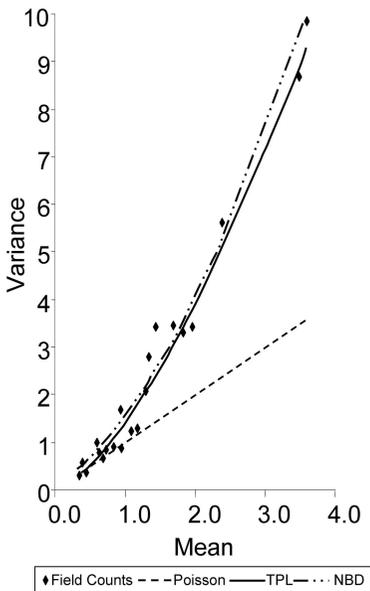
**Fig. 3.** Frequency distribution of sample unit counts for European chafer third instars in a winter wheat field taken during spring 2003 at Charlotte, MI. Bars are the actual count frequency with field mean,  $M = 1.30$ . Circles are the expected negative binomial probabilities using  $M = 1.30$  and  $k = 2.15$  (TPL) as parameters. This site fit the NBD ( $P = 0.8984$ ) but did not fit the Poisson distribution ( $P > 0.0001$ ) by using a chi-square test.

**Table 2.** Frequency distribution models fit to field counts of European chafer third instars and tested for homogeneity using a chi-square statistic

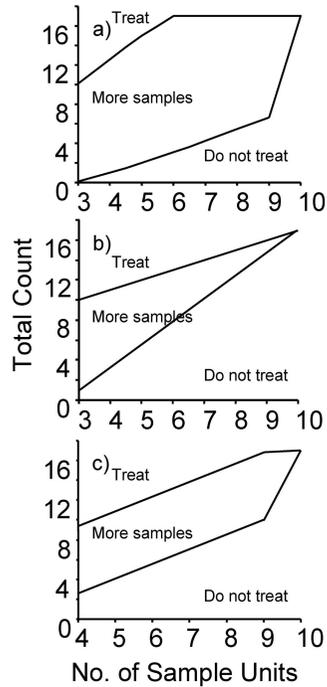
Field mean	k	df	$\chi^2$ Poisson	P value NBD	Distribution best-fit
0.35	-2.69	7	0.9952		Poisson
0.39	-4.18	5	0.4597	0.0814	Both
0.44	-12.82	8	0.9585	0.0735	Both
0.45	-17.22	7	0.9896	0.9910	Both
0.60	5.22	9	0.0278	0.4431	NBD
0.63	4.50	9	0.9059	0.9964	Both
0.69	3.65	9	0.8076	0.8409	Both
0.73	3.30	10	0.8989	0.9999	Both
0.84	2.73	10	0.8820	0.9790	Both
0.94	2.50	10	0.1125	0.9746	Both
0.94	2.49	11	0.7872	0.9996	Both
1.09	2.29	11	0.6706	0.6869	Both
1.18	2.22	11	0.9302	0.9634	Both
1.30	2.16	12	0.2149	0.8854	Both
1.30	2.15	12	<0.0001	0.8984	NBD
1.34	2.14	12	<0.0001	0.8640	NBD
1.44	2.11	12	<0.0001	0.0040	Neither
1.68	2.08	13	0.0312	0.9961	NBD
1.83	2.07	14	0.0527	0.9794	Both
1.96	2.07	14	0.0423	0.7495	NBD
2.39	2.11	15	<0.0001	0.0069	Neither
3.49	2.25	15	<0.0001	0.2923	NBD
3.59	2.27	15	<0.0001	0.7381	NBD

$P > 0.05$  was the significance level for homogeneity between the counts and the Poisson or NBD distributions. Values of k modeled from TPL. One field fit only the Poisson, seven only the NBD, 13 both, and two neither.

lowed similar Poisson and NBD expectation lines, whereas fields with means  $>1.0$  larva per sampling unit were generally closer to the line of NBD expectation.



**Fig. 4.** Relationship between the variance (V) and sample means (M) of European chafer third instars. Each data point is one field in 2002 or 2003 of 18–70 sites each consisting of eight 11-cm-diameter plugs. The dotted line is the Poisson expectation ( $V = M$ ), the solid line is the TPL expectation ( $V = aMb$ ), and dashed line is the NBD expectation ( $V = M + M^2/kc$ , where  $kc = 2.044$ ).



**Fig. 5.** (a) Iwao's sequential classification boundaries, (b) converging lines sequential classification boundaries, and (c) Wald's sequential probability ratio test for making control treatments for European chafer third instars in cornfields. A total larval count falling above the upper boundary requires a treatment, a count falling below the lower boundary does not require a treatment, and a count falling in between the boundaries requires additional sampling. At a maximum sample unit size of 10, a treatment decision is made comparing the total count to a single critical density. Parameters used to calculate the classification boundaries were  $cd = 1.7$ ,  $minn = 3$ ,  $maxn = 10$ ,  $\alpha = 0.10$ ,  $TPL a = 1.42$ ,  $TPL b = 1.47$ , and  $V$  calculated from TPL relationship.

Sequential sampling classification boundaries were calculated using Iwao's procedure (Fig. 5a), a converging lines procedure (Fig. 5b), and Wald's sequential probability ratio test (Fig. 5c). A maximum  $n$  of 10 sample units had a coefficient of variation of 32.8%. Boundaries shown for each procedure are those using parameters resulting in minimal or flat ASN curves without compromising the steepness or accuracy of the OC curve. Larval counts falling above the upper boundary or below the lower boundary at a particular number of sample units results in a decision to treat or not to treat, whereas those counts falling between the two boundaries suggest that there is insufficient information to make a decision, requiring additional samples (Fig. 5).

When OC and ASN functions were compared among the three procedures, accuracy or steepness of the OC function and minimal number or flatness of the ASN function were used as factors to determine the best sequential sampling procedure (Fig. 6a, b). The converging lines procedure required the fewest average samples, but the accuracy of the procedure was

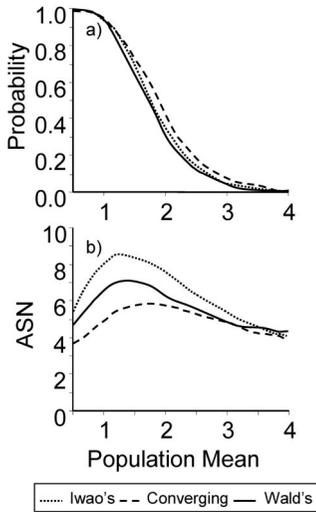


Fig. 6. (a) OC function comparing the accuracy of three sequential sampling plan procedures and (b) ASN required for three sequential sampling procedures for European chafer third instars in corn given a true population mean. For each procedure, 1,000 simulations (sr) drawing random variables from a NBD at a particular population mean was conducted using MathCAD software. Standard error of the OC function can be calculated as  $\sqrt{p(1-p)/sr}$ , where  $p$  is the probability.

less than the other procedures. The accuracy of Iwao's and Wald's procedures was very similar, but Wald's procedure required approximately two fewer samples when the population mean was close to the critical density. Based on these criteria, Wald's sequential probability ratio test was chosen as the best sampling plan.

**Risk Assessment Model.** A forward stepwise regression identified percentage of sand ( $F = 7.72$ ,  $P = 0.0056$ ) and soil bulk density squared ( $F = 24.17$ ,  $P < 0.0001$ ) as significant predictors of larvae density following the form of equation 17:

$$\ln(\text{larval density}) = -1.4914 + 0.0057(\% \text{ sand}) - 0.2052(\text{soil bulk density}^2) \quad [17]$$

The  $C_p$  was  $2.11 \approx P = 2$ ; however, the model only explained 5.4% of the larval density variation. The interaction between percentage of sand and soil bulk density squared was not significant (variance inflation of 1.27) and was not entered into the stepwise regression.

The greatest density of larvae was found at field areas containing a moderate level of percentage of sand and a low soil bulk density (Fig. 7). At all levels of percentage of sand, larval density decreased with increasing soil bulk density (risk categories A to C, D to F, and G to I). At moderate and high bulk density, larval density increased as percentage of sand increased (risk categories B, E, and H and C, F, and I). At low bulk density, larvae density was least at low percentage of sand, greatest at medium percentage of

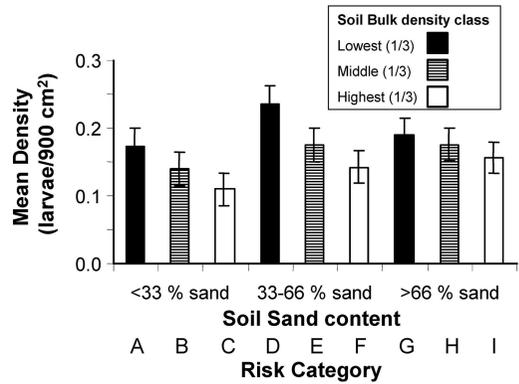


Fig. 7. Mean densities ( $\pm$ SE) of European chafer third instars corresponding to risk values A to I characterized by using percentage of sand and soil bulk density variables. Lowest, middle, and highest third of variable data in each of the 17 fields represented by <33, 33-66, and >66%, divisions. A sampling unit is one golf cup cutter with a diameter of 10.8 cm or 91.4  $\text{cm}^2$  (10 sampling units  $\approx$  900  $\text{cm}^2 \approx$  1  $\text{foot}^2$ ).

sand, but nearly equal for low and high percentage of sand (risk categories A, D, and I).

The critical density is 0.215 larva per sampling unit where the sampling unit was one golf cup cutter with a diameter of 10.8 cm or 91.4  $\text{cm}^2$  (10 sampling units  $\approx$  900  $\text{cm}^2 \approx$  1  $\text{foot}^2$ ). Therefore, risk categories A, D, E, G, and H approach or exceed the critical density. Field areas where the percentage of sand is high and the soil bulk density is low to moderate or where the percentage of sand is moderate and the soil bulk density low should be chosen as sampling locations. However, areas of low percentage of sand and moderate to high soil bulk density should not be chosen as sampling locations.

## Discussion

By demonstrating a relationship between an increasing density of third instars and responses in emerging field corn, a critical density of two larvae 900  $\text{cm}^{-2}$  is suggested for use in control decisions. The critical density should not be used as an economic threshold because the relationship only showed the effects of larval density on 4-wk old corn plants and not on the marketable yield of the plants. However, using the greenhouse and outdoor trials, the number of missing or damaged plants increased  $\approx$ 8% from zero to two larvae per microplot. Assuming a direct linear relationship between missing plants in microplots and yield loss in a field, the 8% loss equates to \$62/ha by using a yield of 7 tons/ha and market price of \$111/ton (Baute 2002). An insecticide cost of \$16.00/ha that is 80% effective (e.g., clothianidin at 20 g [AI]/100 kg seed; A.W.S., unpublished) would net a \$33.60/ha return.

Although a critical density of one larva 900  $\text{cm}^{-2}$ , especially for large fields, justifies the use of clothianidin, it was not lowered for the following reasons. First, only data from two studies were used to calculate

the number of damaged and missing plants in microplots. Variable field and crop characteristics make it difficult to precisely estimate the appropriate critical density to use. Second, although outdoor microplots, with one and two larvae had identical numbers of missing or damaged plants as the controls, the fresh weight of corn plants was lower when plots with zero and two larvae were compared. Although 4 wk approximated the natural feeding time of larvae in corn (Renkema 2004), the probability of feeding for a longer period and its impact on plant growth is uncertain. Finally, field observations of corn populations and larval density over the past 2 yr never show a visual reduction in plant stand below a density of two larvae  $900 \text{ cm}^{-2}$ .

A variance–mean relationship description of the data are more advantageous than a probability distribution model because of its portability (Binns et al. 2000). Although there has been debate about variance–mean relationships (Trumble et al. 1989), the *a* and *b* parameters of the TPL are generally robust across time and space. Differences in crop type, pesticide application, natural enemies, or geographic location may have an effect on the aggregation parameter, *b*. Taylor's *a* (1.15) and *b* (1.07) for European chafer larvae in pastures (Taylor 1961) are very different from values calculated in the current study for larvae in field corn. Difference in sampling unit size ( $900 \text{ cm}^{-2}$  versus eight 11-cm-diameter plugs) may account for the difference in the sampling parameter *a*; however, the difference in *b*, between field corn and pasture, is not as easily explained. The TPL parameters for pasture relied on data from three sites during 1 yr (Burrage and Gyrisco 1954b), whereas the current field corn calculation included 18 different sites over 2 yr. The pasture data consisted of  $n = 75$  means and variances (25 per field), and each mean was calculated from 25 sampling points. The field corn data consisted of  $n = 23$  means and variances and each mean was calculated from a variable number of sampling points depending on the size of the field. Binns et al. (2000) suggested at least 40 sample observations for the calculation of each mean; however, smaller numbers have been used (Taylor et al. 1988). In addition, Taylor (1961) did not report how well the TPL fit the data; the  $R^2$  for the field corn data were 0.94. The range of means in pasture data were large, 0.20–9.32 larvae per square foot. The range of means in the field corn data spanned the critical density, 0.2 larva per golf cutter cup, but only six of 23 means were greater than the critical density. Differences in methods used to sample European chafer larvae may have contributed to differences in the calculation of TPL parameters; however, much of the difference in the *b* value may be simply because of a difference in the distribution or degree of aggregation of the larvae in pasture versus field corn.

The accuracy of TPL parameters in field corn requires validation on additional fields in subsequent years with sampling on a finer scale. This is especially true for smaller fields where the number of samples needs to be at least 30–40. Also, more sampling in

fields where the mean density is above critical density is required so the spread of the data points is greater. Small changes in TPL parameters generally have little effect OC and ASN for evaluation of sampling plans (Binns et al. 2000). Only a large change in the parameters would invalidate the currently proposed sequential sampling plan.

Calculating the loss because of pest density,  $L(\mu)$ , and the prior probability of pest density,  $P(\mu)$ , allows determination of the value of sampling (Binns et al. 2000); however, with the current data, exact calculations were not possible. The relationship between larval density and corn fresh weight and number of damaged or missing plants 4 wk after planting is not predictive of yield. Nevertheless, at low densities of two larvae  $900 \text{ cm}^{-2}$ , between one and six and one and 12 corn plants were lost. Therefore, the cost of sampling should be low to justify the result. Sampling is relatively inexpensive (only a trowel is required) and time-efficient (digging and evaluating eight 1-dm<sup>2</sup> samples took 5–10 min and 10 sites in one field, 1.5 h).

Predicting larval density without sampling is nearly impossible considering the limited information on the distribution and ecology of European chafer in field crops. Environmental conditions such as winter soil temperatures and moisture levels affect yearly larval population levels (Tashiro et al. 1967). Therefore, an equal probability for a wide range of field densities should be assumed at this time and, because the collection of sample information is relatively efficient, annual early samples for larvae before planting corn is recommended.

Percentage of sand and soil bulk density were useful predictors of larval densities in a field; however, they accounted for <6% of the variation in larval density. Larval density decreased with increasing soil bulk density at any level of percentage of sand. Larval density was always lower in areas of low percentage of sand, highly variable for areas of moderate percentage of sand, and consistently higher for areas of high percentage of sand. Based on these results, sampling for larvae is recommended in field areas containing moderate-to-high percentage of sand and low bulk density.

Other variables not measured in the current study may account for differences in larval distribution. The prediction model relies on larval aggregation; however a few fields had a random distribution, which may decrease the effectiveness of the entire model somewhat. Little is known about oviposition preferences for particular field landscapes, and these may affect distribution. Although, all mating occurs on trees and Tashiro et al. (1969) showed higher larvae numbers near mating sites, distance to nearest tree was not predictive in corn fields. Finally, if aggregation is because of differential mortality rates within fields, variables such as moisture content, snow cover, or presence of natural enemies may explain more of the field wide variation and should be considered.

Predetermining areas for sampling with field characteristics introduces bias into the sampling plan, and the estimated mean density is likely different than the actual mean of the entire field (Binns et al. 2000).

However, current control strategies for European chafer in corn (seed treatment) can only be practically applied fieldwide, but the distribution of damage is not. An entire field should be treated if the predicted areas of highest larvae density are above critical density. If a random sampling technique is used, a field may be classified as not needing treatment because the mean population was less than the critical density even though patches of larvae were above critical density levels. Thus, predetermining sampling locations lessens the probability of making an error where treatment is not applied but needed in areas of the field where high densities of larvae occur. In addition, introducing bias into the sampling plan may reduce the cost of sampling (Binns et al. 2000). Fewer samples may need to be processed if samples are taken only where high densities of larvae are predicted.

The usefulness of a control decision guide for European chafer third instars infesting corn depends upon the proper choice of input data, presentation and ease of implementation methods, and evaluation and reevaluation by simulation and feedback from practitioners (Binns et al. 2000). Results presented here largely focus on the input data such as critical density, spatial distribution, and predicting areas of risk, and some evaluation by simulation, the OC and ASN functions. In most respects, these inputs seem accurate and precise; however, corroboration through further field validation is needed.

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