SAMPLING AND BIOSTATISTICS

Spatial Distribution of *Phyllophaga implicita* (Horn) (Coleoptera: Scarabaeidae) Larvae in Relation to Distance from the Adult Food Source

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ABSTRACT The spatial distribution of the *Phyllophaga implicita* (Horn) larval population in relation to the distance from the adult food source was investigated. Commercial soybean fields with 1 of 4 different shelterbelt arrangements were sampled in the fall of 1993, 1994, and 1995 to assess *P. implicita* distribution and density. Larvae were found only in fields with bordering shelterbelts. Ninety percent or more of the specimens were collected within 55 m of the shelterbelts. Exponential models indicated a significant relationship between larval density and distance from the shelterbelts, with densities declining as distance increased. Logistic regression analyses were performed to assess models for predicting the probability of finding larval infestations. A positive association between the probability of finding larvae (π_X) and the distance from the shelterbelt was found. With *P. implicita* having a 3-yr life cycle, the 1993 season was the flight year for the largest of the 3 broods. Sampling guidelines based on higher densities of larvae nearer the shelterbelts are discussed.

KEY WORDS sampling, white grub, corn

IN THE NORTH-central states, larval feeding by *Phyllophaga* spp. have caused economic losses in several types of field crops. Surveys from 1984 to 1986 report *P. implicita* as comprising 99% of the adult scarab beetles captured in cornfields in the southeast region of North Dakota (McLeod and Schulz 1986). Adult dispersal behavior away from host trees, where the beetles feed on foliage, and larval spatial distribution are 2 key components of *P. implicita* biology that need to be understood before the development of a sampling and subsequent management program.

In many cases, scarab beetles emerge close to the adult host trees because of the egg-laying habits of females (Forbes 1916, Sweetman 1927, Fattig 1944, Fleming 1976, Régnière et al. 1983). Female *P. prununculina* (Burmeister), *P. luctuosa* Horn, and *P. foresteri* Burmeister fly 30 to 90 m away from the host trees before burrowing into the soil to oviposit (Johnston and Eaton 1939). Larval densities were 2 times greater in fields located within 0.3 km of host trees when compared with fields >0.3 km from trees (Forbes 1916). Sweetman (1927) reported 66% of the fields with adjacent host trees were infested by *P. implicita* grubs while 75% of the fields greater than 0.3 km from trees were not infested. Others have concluded that a high risk of infestation exists under continuous crop plantings when fields are near the adult food sources (Lentz 1985, Kard and Hain 1988). *P. implicita* adults feed on the foliage of willow (*Salix* spp.), poplar (*Populus* spp.), ash (*Fraxinus* spp.), and elm (*Ulmus* spp.) (Lago et al. 1979). Shelterbelts bordering fields in southeastern North Dakota are largely composed of these species.

Vertical soil migration by larvae must be considered when developing accurate larval sampling plans for management decisions. In general, larvae begin their upward soil migration in the spring and downward migration in the fall coincident with overturns in soil temperature (McColloch and Hayes 1923, Travis 1939). *Phyllophaga anxia* (LeConte) has been found from 20 to 61 cm deep in the soil profile (Guppy and Harcourt 1973). Observations on larval feeding behavior indicate actively feeding grubs will be in the top 7 cm of soil (Hammond 1944). *Phyllophaga implicita* cease feeding when soil temperatures decrease to <15 to 16°C (Sweetman 1931). Mortality of third-instar *P. implicita* is 100% when they overwinter above the frost line.

After oviposition and egg hatch, it is required to evaluate the larval population density to determine if a management intervention is necessary. The most common threshold densities are 1 larva per 0.09 m^2 or less depending on the annual crop planted (Hammond 1940, 1960; Teetes 1973; Teetes and Sterling 1976).

Sequential sampling programs have been developed for rapid classification of larval populations (Ives and Warren 1965; Teetes and Sterling 1976). Application of similar sequential sampling models for control decisions for *P. implicita* may have problems if adult host plants have an influence on the location within a field where eggs are oviposited. When damaging populations of larvae occur only in portions of a site, sampling throughout the field may detect an overall mean den-

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sity that is below the damage threshold, making mean density an unsuitable parameter on which to base control decisions (Nyrop et al. 1995).

Upon dispersal, gradients develop away from the area of concentration that can be described by empirical models that are logarithmic forms of curves (Freeman 1977; Taylor 1978). The models quantify the slope of the dispersal gradient using occurrence (Y) as the dependent variable plotted against distance (X) from the source. The models are characterized by a sharp peak and long tails. The distributions arise due to differences in a population's capacity to disperse, influenced by the size of the starting population or its ability to disperse (e.g., flight behavior) (Taylor 1978).

Logistic regression can be used to describe the probability of 2 outcomes based on a relationship of these probabilities with 1 or more independent variables. Logistic regression (Hosmer and Lemeshow 1989, Kleinbaum 1994) was used to develop the model. Key features that distinguish logistic regression from linear regression are that the outcome variable is binary and model parameters are best estimated via maximum likelihood (Agresti 1990). A sample is assigned 1 of the 2 binary values based on predetermined criteria. This allows estimation of the probability of an event for a given X, symbolized by π_X. Each estimate of π_X is made by Y_i/N_i, where Y_i = number of samples with activity and N_i = total number of samples observed at point X. The curvilinear relationship of π_X and X is defined as:

$$\pi_x + \frac{\exp^{\alpha + \beta X}}{1 + \exp^{\alpha + \beta X}} \qquad [1]$$

when $X \rightarrow \infty$; the π_X approaches 0 when $\beta < 0$; and π_X approaches 1 when $\beta > 0$.

The linear form of the model is written as:

$$\log\left[\frac{\pi_X}{1 - \pi_X}\right] + \alpha + \beta X \qquad [2]$$

The goal of logistic models is to explore the relationship between π_X as a function of X. The independent variables, X_i ($i = 1, 2, 3,..., n$), can be continuous (i.e., measurements) or discrete (i.e., counts). The value of π_X always ranges between 0 and 1, describing a probability or risk of an outcome. The probability of having larvae present is defined as π_X and none present as $1\text{-}\pi_X$.

There are 2 methods of estimating π_X: the method of maximum likelihood, and the method of weighted least squares. The likelihood of the data and the first order derivatives of the likelihood with respect to α and β are nonlinear functions of α and β. With the method of maximum likelihood, an iterative procedure is used to estimate α and β. Alternatively, the random variable $\ln(p_X/1 - p_X)$ could be used and find that the expected value is approximately $\ln(\pi_X/1 - \pi_X) = \alpha + \beta X$, where p_X is the observed frequency of having larvae present at the distance X. Even though $\ln(p_X/1 - p_X)$ has an expected value which is a linear function of X, its variance is not a constant. The method of weighted least squares could be used to counteract non-constant variance. For this study, the method of maximum likelihood was used to estimate the parameters within the PROC logistic procedures (SAS Institute 1990).

The potential application of logistic regression to larval sampling for *P. implicita* is its modeling ability based on the classification of samples as infested or not infested rather than making total larval counts of all samples. Samples are simply scored as 0 or 1, respectively. For a set of samples, the number with *P. implicita* (i.e., 1) are summed and divided by the total number of samples (probability of finding a larva). If a predictable relationship exists between the probability of finding larvae and the sample distance from shelterbelts, a sampling protocol for determining the need for treatment can be developed. Instead of treating entire fields to prevent larval feeding injury, areas where larval populations exceed the treatment threshold, could be targeted for treatment. It is possible to sample entire sites to locate these zones, but the effort is expected to be timely and costly. Development of a sampling strategy that would identify potential areas of high risk and minimize the effort and costs of evaluating that level of risk would be beneficial.

The purpose of this study was to examine the spatial distribution of the *P. implicita* larval population within production-sized fields in relation to the distance from the adult food source. Logistic regression models were used to describe this relationship and evaluated for their ability to describe the spatial distribution of larvae and for their predictive value in contributing to larval pest management decisions.

Materials and Methods

Commercial soybean fields, ranging in size from 24 to 40 ha, were selected from areas in Richland County in SE North Dakota, where infestations by *P. implicita* have been reported in the past. Selected fields had 1 of 4 different shelterbelt arrangements around the outside perimeter: a single side, adjacent sides (i.e., shelterbelts intersect at one corner of the field), opposite sides, or no sides (nearest trees were >0.81 km). Two new fields were selected and sampled for each arrangement during the fall of 1993, 1994, and 1995. The sampling unit was 0.09 m^2 of soil to a depth of 30 cm (Burrage and Gyrisco 1954), removed and inspected in two 15-cm increments, for a total volume of 0.03 m^3. Samples were dug using an 18-cm wide, straight-edged garden spade. Soil was inspected at the sample site for the presence of larvae, pupae, and adult *Phyllophaga* spp. All specimens were collected, counted, classified according to life stage, and identified to species.

Fall Sampling. Before sampling, field dimensions were estimated to allow for equal spacing of sample sites. A total of 72 samples was taken per field, divided among 6 transects running the width of the field with 12 equally spaced samples per transect. This sampling method allowed for the estimation of distance from the nearest shelterbelt and grouping the subset of samples used to estimate the probability of finding a larva at distance *X*. Fields were sampled during September to the first week of October.

Summer Sampling. Four of the 6 fields studied in the fall of 1993 were planted to corn in April and May of 1994. Five of the 6 fields sampled in the fall of 1994 were planted to corn in May of 1995. Within these fields, areas were left untreated with granular insecticide for conducting follow-up surveys for the presence of larvae. Untreated plots, numbering from 16 to 18, were established at 9-m intervals away from the shelterbelt. Plots were a minimum of 3 rows wide (229 cm by 40 m long). Four randomly selected samples were taken from the middle row of each plot. Sampling was conducted from 15 June to 15 July in both seasons.

Statistical Analysis. Larval density found in samples was analyzed as the dependent variable against distance from shelterbelts for each field and year. Model equations were obtained using PROC REG and PROC NLIN methods in SAS (SAS Institute 1990) for linear and nonlinear regression, respectively. Regression coefficients were tested for homogeneity within years (Gomez and Gomez 1984). Slope (β) and intercept (α) parameters from linear regression equations determined for the combined models by years were compared using a *t*-test. Logistic regression procedures were used to obtain models for predicting the probability of finding at least 1 larva per 0.09 m^2 at a specified distance from the shelterbelts. For this procedure, samples were classified by the presence or absence of larvae. Using a treatment threshold of 1 larva per 0.09 m^2 (Hammond 1960, Teetes 1973, McLeod et al. 1986), a sample where at least 1 larva is found is assigned a value of 1 (= infested); if no larvae are found, then it is assigned the value of 0 (= not infested). The probability of finding larvae at each distance from the shelterbelt was obtained by calculating the ratio, Y/N, where Y = number of samples with at least 1 larva per 0.09 m^2 at distance X and N = total number of samples at distance X. By sampling at a given distance, X, the probability of having a population level of larvae at the treatment threshold can be determined. These ratios were regressed against their distances using PROC LOGISTIC in combination with PROC CATMOD (SAS Institute 1990). Estimated slopes (β) and intercepts (α) from the logistic regression equations from different shelterbelt arrangements and years were compared using a *t*-test (Little and Hills 1978).

Results

Phyllophaga implicita were found only in fields associated with shelterbelts. Larvae were found 97% (1993), 100% (1994), and 95% (1995) of the time in the top 15 cm of soil. The mean density for individual fields ranged from 0.1 to 2.3 larvae per 0.09 m^2 over the 3 yr (Table 1). All larvae collected during 1993 were 1st or 2nd instars. In 1994 and 1995, 2nd and 3rd instars were found. In general, the numbers of larvae found were considerably fewer in 1994 and 1995 than in 1993. The 1993 season was a major flight year for *P. implicita*, accounting for the larger number of larvae found.

Table 1. Mean density of *P. implicita* per 0.90 m^2 and maximum number of specimens found in a single sample in soybean fields with different shelterbelt arrangements in Richland County, North Dakota

Shelterbelt arrangement and field	1993		1994		1995	
	Mean ± SEM	Max	Mean ± SEM	Max	Mean ± SEM	Max
			per 0.09 m^2			
Adjacent I	1.1 ± 0.3	13	0.3 ± 0.1	4	0.2 ± 0.05	2
Adjacent II	0.2 ± 0.1	5	0.1 ± 0.04	2	0.0 ± 0.01	1
Opposite I	0.5 ± 0.1	7	0.1 ± 0.04	2	0.2 ± 0.1	5
Opposite II	2.3 ± 0.4	16	0.2 ± 0.1	2	0.2 ± 0.1	2
Single I	0.3 ± 0.1	5	0.2 ± 0.1	3	0.1 ± 0.03	1
Single II	1.0 ± 0.2	11	0.1 ± 0.03	2	0.1 ± 0.04	2

Adjacent shelterbelts intersect at one corner of the field; opposite shelterbelts were on opposite sides of the field; single shelterbelt was present on only one side of the field. Samples totaled 72 from each field.

Based on the overall mean density of larvae per 0.09 m^2, only 3 fields, all sampled in the fall of 1993, exceeded the recommended treatment threshold of one larva per 0.09 m^2. However, larval densities were greater near the shelterbelts. In all fields, ≥90% of the *P. implicita* specimens were collected within 55 m of the shelterbelts (Fig. 1). Ninety-nine percent or more of the larvae were found within 118 m of the shelterbelts. In 1993, larval densities near shelterbelts exceeded recommended treatment thresholds in all 6 fields with shelterbelts.

Larval densities declined with increased distance from the shelterbelts. Linear forms of the exponential model, where log transformations of larval density were regressed against distance, indicated significant relationships between density and distance at all field sites in 1993, four of 6 sites in 1994, and only three of 6 sites in 1995 (Table 2). Regression coefficients within years were homogeneous (1993: $F = 2.33$; df =

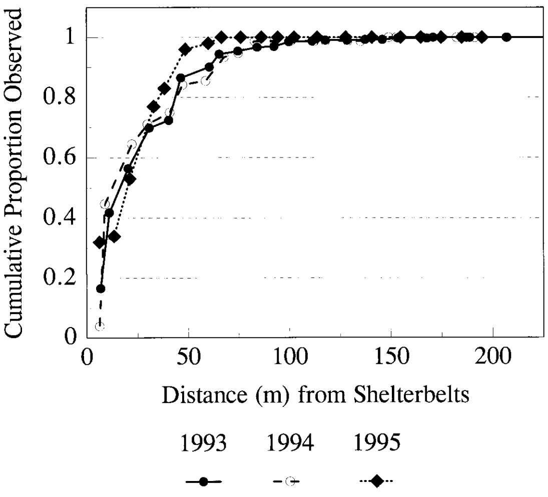

Fig. 1. Cumulative proportion of *P. implicita* specimens collected during fall sampling at increasing distances from shelterbelts bordering soybean fields.

Table 2. Nonlinear regression parameters relating larval densities found during fall sampling to distance from bordering shelterbelts

Shelterbelt arrangements and field	Model parameters[a]		F[b]	r^2[b]
	α ± SEM	β ± SEM		
	1993			
Adjacent I	8.62 ± 1.27	−0.035 ± 0.006	17.57*	0.59
Adjacent II	2.29 ± 0.31	−0.069 ± 0.012	10.75**	0.47
Opposite I	3.06 ± 0.12	−0.032 ± 0.002	26.34**	0.81
Opposite II	6.19 ± 1.28	−0.029 ± 0.009	18.35*	0.82
Single I	2.37 ± 0.52	−0.038 ± 0.011	7.19*	0.41
Single II	6.62 ± 0.86	−0.032 ± 0.006	37.33**	0.79
All sites	4.90 ± 0.24	−0.034 ± 0.002	65.05**	0.50
	1994			
Adjacent I	3.46 ± 0.33	−0.049 ± 0.006	10.49*	0.49
Adjacent II	1.58 ± 0.47	−0.073 ± 0.026	7.86*	0.47
Opposite I	0.84 ± 0.19	−0.078 ± 0.021	5.32	0.57
Opposite II	0.34 ± 0.28	−0.013 ± 0.024	0.83	0.17
Single I	1.74 ± 0.27	−0.038 ± 0.008	20.25**	0.67
Single II	0.37 ± 0.14	−0.034 ± 0.018	5.15*	0.34
All sites	0.74 ± 0.16	−0.028 ± 0.008	33.37**	0.36
	1995			
Adjacent I	0.54 ± 0.16	−0.021 ± 0.009	15.97**	0.59
Adjacent II	ND	ND	3.13	ND
Opposite I	0.31 ± 0.20	−0.014 ± 0.019	2.33	0.37
Opposite II	0.47 ± 0.18	−0.033 ± 0.019	5.14	0.56
Single I	0.46 ± 0.06	−0.070 ± 0.011	12.64**	0.56
Single II	0.50 ± 0.15	−0.046 ± 0.019	9.82*	0.50
All sites	0.32 ± 0.21	−0.027 ± 0.008	36.17**	0.38

Adjacent shelterbelts intersect at one corner of the field; opposite shelterbelts were on opposite sides of the field; single shelterbelt was present on only one side of the field. ND, not determined.

[a] Model parameters are for nontransformed data using the nonlinear exponential model, $Y = \alpha \exp^{(\beta X)}$.

[b] F and r^2 are for the linear form of the exponential model, $\mathrm{Ln}\ Y = \mathrm{Ln}\ \alpha + \beta X$. Asterisks indicate that the linear regression model was significant at $P < 0.05$* or $P < 0.01$**.

5, 54; not significant; 1994: $F = 1.64$; df = 5, 50; not significant; 1995: $F = 0.47$; df = 5, 50; not significant). When data were combined from all fields within 1 yr, a significant functional relationship was obtained (Table 2). Overall, larval densities were lower in both 1994 and 1995 when compared to 1993. The slope parameters from the combined field models were not significantly different among years. The intercept, α, for the 1993 combined model was significantly different from those in the 1994 and 1995 combined models ($t = 14.4$, $t = 16.4$, respectively); α was not significantly different between 1994 and 1995 ($t = 1.6$), so computation of a common model was not attempted. The main difference for the 3 yr is overall density of larvae. The main component of populations from all three years is the *P. implicita* brood which hatched during the 1993 season.

During follow-up sampling in summers of 1994 and 1995, larvae were found in the same field areas as in the fall of 1993 and 1994 (Figs. 2 and 3). In 1994, mean summer densities were generally lower (Table 3) averaging 42% fewer larvae from the fall of 1993. Mean densities in the summer of 1995 averaged 54% fewer larvae from the fall of 1994. Summer densities remained higher nearest the shelterbelts.

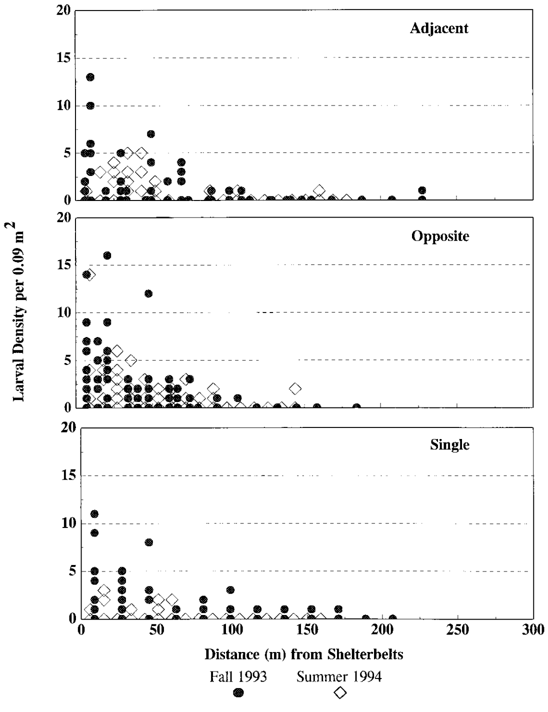

Fig. 2. Larvae of *P. implicita* found in the fall of 1993 and the summer of 1994 from the same fields with either adjacent, opposite, or single shelterbelts.

Logistic regression was performed on the estimated probabilities for finding at least 1 larva per 0.09 m^2 and sample distances from shelterbelts. Some models for the different shelterbelt arrangements from each year had significant chi-square values indicating a positive association between the probability of finding larvae (π_X) and the distance, X, from the shelterbelt (Table 4). Using t-tests ($P < 0.05$) to compare among slope (β) and among intercept (α) parameters, there were no significant differences between models for the different shelterbelt arrangements within years. Logistic regression models based on the combined observations from all sites by year were determined (Table 4). The combined models (Figs. 4, 5 and 6) had significantly different intercepts among years so that computation of a common model was not attempted (1993 versus 1994, $t = 4.36$*; 1993 versus 1995, $t = 5.37$*; 1994 versus 1995, $t = 1.21$; *, significantly different at $P < 0.05$).

All fields sampled in the fall of 1993 had larval densities close to shelterbelts that exceeded the treatment threshold of one larva per 0.09 m^2. Overall densities and the distances from the shelterbelts at which densities fell below the threshold were different for each field. In 1994, only 2 sampled fields had larval densities close to shelterbelts that exceeded the treatment threshold. All larvae were 3rd instars and not expected to cause significant feeding injury the following year (Hammond 1948). No fields sampled in 1995 had larval densities exceeding the treatment threshold.

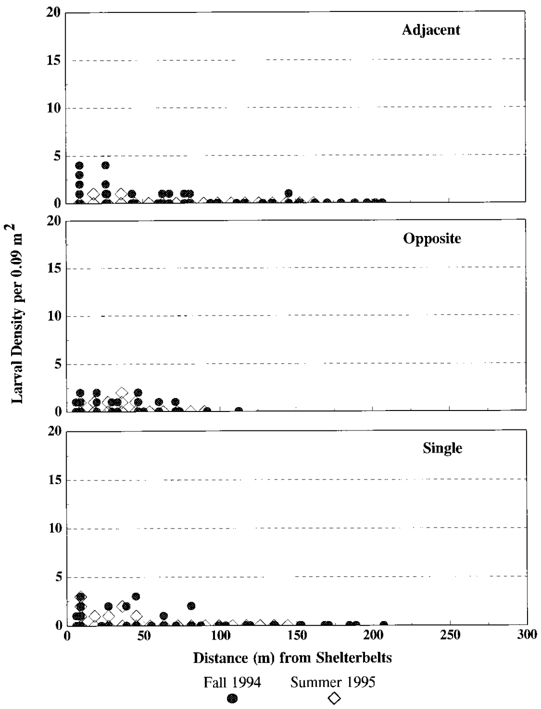

Fig. 3. Larvae of *P. implicita* found in the fall of 1994 and the summer of 1995 from the same fields with either adjacent, opposite, or single shelterbelts.

Discussion

Densities of *P. implicita* larvae were generally greatest near shelterbelts used by the adults as their food source. Distance from the shelterbelts at which larval populations fell below 1 larva per 0.09 m^2 varied from field to field, but did not extend beyond 90 m in any field sampled during this study. Sampling programs can be limited to this area in a field for evaluating populations of *P. implicita.*

Logistic regression models demonstrated a relationship between the probability of finding infested samples and distance from shelterbelts. Probabilities declined with increasing distance. Over the 3 years of this study, when $\pi_X = 0.55$, a larval density of 0.94 larvae per 0.09 m^2 would be predicted, near the treatment threshold of one per 0.09 m^2 (Fig. 7). This regression model uses one variable that is normally distributed, mean number of grubs per 0.09 m^2, and another which is a binary variable, π_X , and therefore not normally distributed, so the relationship is weak. However, the relationship does strengthen the support of using π_X as an indicator of larval activity when a series of samples is taken at a specified distance from a shelterbelt.

Table 3. Mean ± SEM density of *P. implicita* per 0.09 m^2 in the summer and percent change in the mean from the previous fall from fields with different shelterbelt arrangements in Richland County, North Dakota

Shelterbelt arrangements and field	Summer, 1994		Summer, 1995	
	Mean ± SEM	% change	Mean ± SEM	% change
Adjacent I	0.7 ± 0.2	−36	0.03 ± 0.02	−90
Adjacent II	ND	ND	ND	ND
Opposite I	0.2 ± 0.1	−60	0.09 ± 0.04	−10
Opposite II	1.3 ± 0.3	−40	0.06 ± 0.03	−70
Single I	0.2 ± 0.1	−33	0.20 ± 0.07	0
Single II	ND	ND	0.00 ± 0.00	−100

Adjacent shelterbelts intersect at one corner of the field; opposite shelterbelts were on opposite sides of the field; single shelterbelt was present on only one side of the field. ND, not determined.

Table 4. Logistic regression[a] parameters relating the probability (π_X) of finding an infested sample relative to distance (*X*) from shelterbelts during fall sampling

Model source	Regression parameters		Likelihood ratio X^2	*P*	d. f.
	α ± SEM	β ± SEM			
Year–1993					
Single	1.13 ± 0.42	−0.024 ± 0.005	9.26	0.507	10
Adjacent	0.89 ± 0.44	−0.036 ± 0.008	40.95	0.017	24
Opposite	2.00 ± 0.42	−0.036 ± 0.007	16.34	0.090	10
Combined	1.31 ± 0.23	−0.033 ± 0.004	79.12	0.003	48
Year–1994					
Single	0.14 ± 0.54	−0.046 ± 0.014	14.20	0.894	22
Adjacent	0.40 ± 0.48	−0.034 ± 0.008	17.21	0.752	22
Opposite	−0.60 ± 0.43	−0.034 ± 0.011	16.14	0.096	10
Combined	−0.20 ± 0.26	−0.034 ± 0.006	45.33	0.335	42
Year–1995					
Single	−0.41 ± 0.55	−0.067 ± 0.022	4.41	0.927	10
Adjacent	−0.51 ± 0.53	−0.040 ± 0.013	5.39	0.911	11
Opposite	−0.87 ± 0.46	−0.035 ± 0.013	5.25	0.812	9
Combined	−0.67 ± 0.29	−0.043 ± 0.008	17.01	0.990	33

[a] $\log\left[\frac{\pi_X}{1-\pi_X}\right] = \alpha + \beta X.$

Adjacent shelterbelts intersect at one corner of the field; opposite shelterbelts were on opposite sides of the field; single shelterbelt was present on only one side of the field.

Larval populations of *P. implicita* would seldom be expected to to exceed treatment thresholds beyond

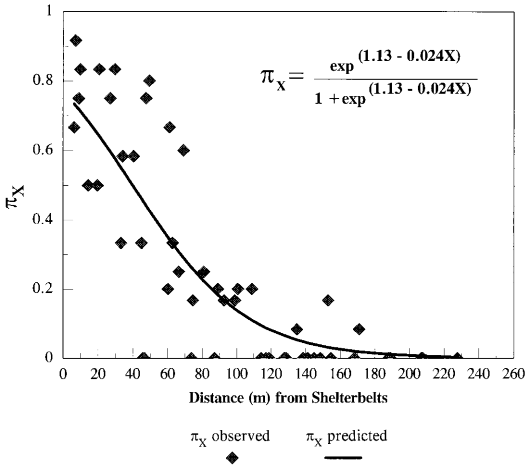

Fig. 4. Probability (π_X) of finding at least 1 larva per 0.09 m^2 at distance *X* from shelterbelts bordering soybean fields based on all fields sampled in the fall of 1993.

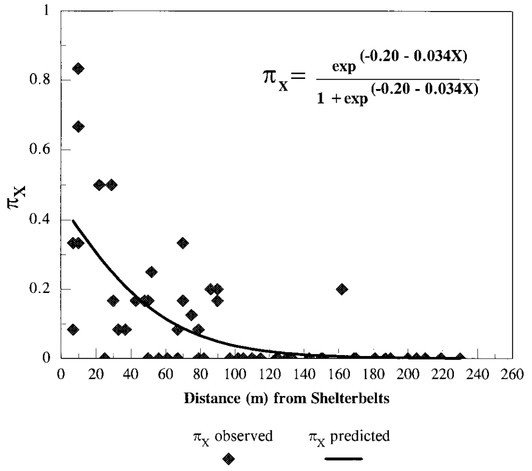

Fig. 5. Probability (π_X) of finding at least 1 larva per 0.09 m^2 at distance X from shelterbelts bordering soybean fields based on all fields sampled in the fall of 1994.

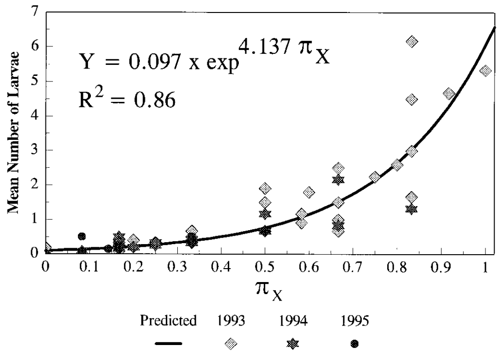

Fig. 7. Relationship between the mean number of *P. implicita* found per 0.09 m^2 and the probability (π_X) of finding at least 1 larva per 0.09 m^2.

90 m from shelterbelts. Field sampling to determine the need to apply insecticides should take place within this 90-m zone. When using a series of samples at a given distance, a presence-abscence sampling plan may be valuable, limiting the need to count the total number of larvae in a given soil sample. Because larvae are normally found in the top 15 cm, they could be discovered with the first shovel of soil. In this study, the time to process the entire 0.03 m^3 of soil ranged from 6 to 18 min, averaging 9 min per sample for an entire field. Processing time increased when specimens were found because of the need to collect and record samples. Further, there is little need to sample below a depth of 15 cm to search for larvae, reducing the total soil volume requiring inspection. Sampling should not be carried out after a killing frost has occurred in the region. Larvae will have moved down in the soil profile for overwintering below the frost line which makes sampling unreliable. Late summer and early fall are the best times for sampling because larvae are actively feeding near the soil surface. Spring sampling, as recommended for North Dakota (McLeod et al. 1986), has not been reliable in the region because planting may occur before a large proportion of the overwintering larvae have reached the uppermost soil layers where they can be observed. Fall evaluation would permit greater planning by the producer in selecting cropping options and insecticide use requirements.

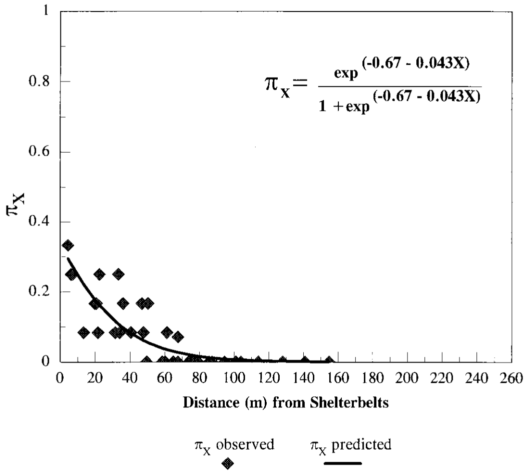

Fig. 6. Probability (π_X) of finding at least 1 larva per 0.09 m^2 at distance X from shelterbelts bordering soybean fields based on all fields sampled in the fall of 1995.

Acknowledgments

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