Binomial Sampling Plans for Tentiform Leafminer (Lepidoptera: Gracillaridae) on Apple in Utah

VINCENT P. JONES

Department of Biology, Utah State University, Logan, Utah 84322-0315

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ABSTRACT The dispersion of Phyllonorycter elmaella Doganlar & Mutuura (Lepidoptera: Gracillaridae), a tentiform leafminer infesting apple in Utah, was investigated over a 3-yr period. According to Taylor's power law, mines are only slightly clumped on a per leaf basis ($\alpha = 1.14$, $\beta = 1.05$, $r^2 = 0.94$). Two different binomial sampling plans based on a constrained negative binomial distribution were evaluated to estimate populations of leaf miners near the one to three mines per leaf economic threshold proposed for other leafminers of this genus attacking apple in the eastern United States. The proportion of leaves infested could not provide accurate predictions over 1.7 mines per leaf; however, the proportion of leaves infested with two or more mines predicted levels above three mines per leaf accurately. The use of this sampling plan in pest management programs is discussed.

KEY WORDS Insecta, Gracillaridae, leafminers, sampling

Tentiform leafminers in the genus Phyllonorycter (Lepidoptera: Gracillaridae) have become increasingly important pests of apple orchards since the 1970s. Their increase has occurred concurrently with reports from many apple-producing areas of the development of resistance to organophosphate materials used for control of other pests such as the codling moth, Cydia pomonella (L.) (Lepidoptera: Tortricidae) (Pree et al. 1980, 1986; Weires et al. 1982). Although these insects are still susceptible to pyrethroids and the oxime carbamate oxamyl in most areas (Pree et al. 1986), these pesticides are disruptive to natural enemies of spider mites when high application rates or multiple applications are used.

At least three species of Phyllonorycter leafminers found in North American apple orchards are considered to be ecological homologs in terms of general phenology, niche in the ecosystem, and type of damage (Hoyt et al. 1983). Feeding by the first three instars is generally confined to the leaf’s spongy mesophyll (sap-feeders), while feeding by the last two instars is extended to the upper palisade cells (tissue-feeders) (Gibb 1984). Feeding in the latter two instars results in a tent-shaped mine with spots where the tissue directly beneath the epidermis has been removed, thus giving rise to the common name “spotted tentiform leafminer” for *P. blanchardella* (F.) and “tentiform” leafminer for other species in the genus.

In Utah, *P. elmaella* Doganlar & Mutuura has up to four generations per year (Gibb 1984). The overwintering generation emerges in early spring, often around the green tip stage of apple phenology. The second generation peaks in late May or early June, the third generation in late July or early August, and the fourth generation near the later part of September if the weather remains favorable. Population trends between years or even between generations within a single year vary markedly, probably because of the effectiveness of at least six parasitoid species which attack *P. elmaella* (Barrett & Jorgensen 1986).

Economic thresholds for *P. blanchardella* have been set at one to three mines per leaf (Weires 1977, Prokop et al. 1980). Integration of *P. elmaella* management tactics into present IPM programs could be greatly facilitated if methods were available to quickly determine population levels within an orchard with respect to these thresholds. Pheromone traps with adhesive-coated bottoms are poor tools for monitoring these insects because they become saturated in less than an hour in heavily infested orchards. Even when nonsaturating traps are used (Vincent et al. 1986), there is no method of relating larval damage to the number of adults captured. Leaf samples are relatively easy to take, but may require more time to process than pest managers are willing to spend. This study investigates the dispersion of *P. elmaella* on apple in Utah and examines the feasibility of developing binomial sampling plans which can be used to quickly estimate populations relative to the threshold previously proposed for *P. blanchardella*.

Materials and Methods

**Sampling Procedures.** A total of 14 orchard samples were taken in commercial apple orchards in Box Elder and Cache counties, Utah, between
June 1986 and October 1988. Apple varieties were 'Red Delicious' in half of the orchards sampled, with the other orchard samples consisting of a mixture of 'Red Delicious,' 'Rome Beauty,' and 'Jonathan' apples. All samples were taken during the second through fourth generations of *P. elmaella*. An orchard sample was composed of between 15 and 40 trees which were randomly selected within an orchard. Each tree was sampled by randomly selecting leaves from around the periphery of each tree between 1.2 and 2 m from the ground and between 0 and 1 m into the canopy. In 1986 and 1987, 40 leaves per tree were collected and in 1988, 20 leaves per tree were collected. All leaves from a single tree were put into a paper bag which was placed in an ice chest and taken to the laboratory. The numbers of sap and tissue mines were recorded separately for each leaf on a tree. A total of 399 trees were sampled in this fashion over the 3-yr study.

**Analysis.** The validation data set was chosen by randomly selecting one orchard sample per year (20, 25, and 40 trees in the orchard samples selected during 1986, 1987, and 1988, respectively) from the total of 14 orchard samples. The data set used for sampling plan development was composed of the remaining 11 orchards (314 of 399 total trees). For development of the sampling plan, the total number of mines per leaf was initially used for the analysis. However, to obtain a wider range of means for both regression analysis and the validation study, the number of larvae in the tissue and sap feeding stages, as well as the total number of mines were used. This approach seemed reasonable because the dispersion of the mines did not change as they developed (e.g., all tissue mines were at one time sap mines) and regression parameters changed little between the different methods of analysis.

Dispersion analysis was performed using Taylor's power law (Taylor 1961), which is defined as

$$s^2 = am^\beta,$$

(1)

where \(s^2\) = sample mean and the coefficients \(a\) and \(\beta\) are described by Taylor as "species specific" parameters. For this study, the coefficients were obtained by performing a log-log transformation and using linear regression (Wilkinson 1986) to estimate \(a\) and \(\beta\) (i.e., \(\log s^2 = \log a + b \log m\), where \(a\) and \(b\) are sample estimates of \(a\) and \(b\)). The \(\beta\) parameter was considered by Taylor to be a measure of clumping; if \(\beta < 1\) the population was uniformly distributed, if \(\beta = 1\) the population was randomly distributed, and if \(\beta > 1\) the population was aggregated. A t test was used to determine if \(\beta\) was significantly different from one. The \(a\) parameter was considered to be related to sample size and the method of sampling (Taylor 1970). However, it should be noted that \(a\) and \(\beta\) contribute to the estimate of population variance (via equation 1) and hence, the dispersion of the species in question is actually a function of both parameters (Wilson 1985).

**Binomial Sampling Models.** Two methods based on the negative binomial distribution were used to develop binomial sampling plans. The basic model could be obtained from the equation

$$P(0) = \left(1 + \frac{m}{k}\right)^{-k},$$

(2)

where \(P(0) = \) proportion of leaves uninfested, \(m\) = mean, and \(k\) is the parameter which is defined as

$$k = \frac{m^2}{(s^2 - m)},$$

(3)

and is considered to characterize clumping as \(k^{-1}\) (Bliss & Fisher 1953). If \(k^{-1} > 0\) then the population was considered to be aggregated, \(k^{-1} = 0\), random and \(k^{-1} < 0\) uniform (Kuno 1986). The values of \(k\) could be obtained by maximum likelihood, variance–mean relationships, or from \(P(0)\)-mean relationships (Bliss & Fisher 1953, Wilson & Room 1983).

Wilson & Room (1983) developed an equation which assumes there is a dynamic link between the mean and variance (i.e., equation 1) that is used to generate the \(k\) of the negative binomial via the relationship

$$k = \frac{m}{(am^{\beta-1} - m)},$$

(4)

where \(a\), \(\beta\) and \(m\) are defined as in equation 1. This results in a negative binomial distribution whose variance is constrained to follow Taylor's power law (Kemp 1987). Wilson & Room (1983) further showed that by combining equation 4 and equation 2, the relationship between the mean population level and \(P(0)\) is

$$P(0) = \exp[-m \cdot \log((am^{\beta-1})/(am^{\beta-1} - 1)).]$$

(5)

For ease of use, \(P(1)\) (the proportion infested \([1 - P(0)]\) was frequently used rather than \(P(0)\).

The second binomial method is an extension of Wilson & Room's procedure so that higher means can be estimated using a presence or absence of more than a given number of individuals per leaf. It is based on the predicted number of leaves (from the constrained negative binomial) which have more than one, two, and so on, individuals per leaf. The expected probabilities for these other frequency classes are given by

$$P(x) = \frac{(k + x - 1)mk^{-1}/(1 + mk^{-1})}{x} \cdot P(x - 1),$$

(6)

where \(k\) is calculated by equation 4, \(m\) is the mean, and \(x\) is the number of organisms found on each observational unit (i.e., if the probability of finding leaves with only one individual present with a given \(m\) and \(k\) is desired, solve for \(x = 1\)) (Bliss & Fisher 1953). The probability of finding leaves with more than one individual is therefore

$$P(>1) = 1 - [P(0) + P(1)].$$

(7)
This sort of expansion can be used to calculate the probabilities of presence or absence of greater than any $x$ number of individuals occurring per sampling unit. The utility of this procedure is that confidence intervals associated with presence–absence sampling became quite large when $P(I) > 0.80$ (Southwood 1978, Jones & Parrella 1986). If the species in question happened to have the desired threshold above $0.80$ using $P(I)$, binomial sampling could still be used if the presence or absence of some easily recognized unit (e.g., more than one or two mines per leaf) is substituted for $P(I)$. Using this method, the prediction of higher means becomes more stable, and the usefulness of presence–absence predictions is extended. For the purposes of this study, the expansion was only run for $P(I > 1)$.

Confidence limits about the prediction can be obtained from the equation

$$CI = \sqrt{\frac{(1 - P(I)) \cdot P(I)}{N}} \cdot Z_{n/2}$$  \hspace{1cm} (8)

where $CI$ = width of the confidence interval, $N$ is the sample size, and $Z = \text{the normal deviate at the desired probability level}$ (Zar 1974).

The fit of the models was assessed using $\chi^2$ analysis to compare the predicted number of leaves infested (equation 3 or 5 multiplied by the number of leaves sampled) and that actually observed in the sample. When predicted frequencies were less than five, the values were not included in the analysis to prevent bias (Zar 1974). In addition, when $P(I)$ or $P(I > 1)$ values were $> 0.95$, values were excluded because these values would not be used in binomial sampling.

The number of trees to sample per orchard necessary to determine the mean levels of infestation within one mine per leaf were obtained from the formula given by Karandinos (1976):

$$N = \left(\frac{Z}{h}\right)^2 \cdot s^2,$$ \hspace{1cm} (9)

where $s^2$ is replaced by equation 1, $Z$ is the normal deviate at the desired level of probability (set at 0.10 in this study), $h$ is the desired width of the confidence interval, and $N$ is the number of trees necessary to sample.

**Results and Discussion**

**Dispersion Analysis.** Taylor’s power law revealed that the within-tree populations were only slightly clumped ($a = 1.14$, $b = 1.05$, $r^2 = 0.94$, $N = 884$). Although $b$ was statistically greater than one ($t = 4.36$, df = 882, $P < 0.001$), biologically, the low $b$ and $a$ values suggest that adult female leafminers lay their eggs randomly throughout the tree.

The between-tree $s^2/m$ relationship was well described by Taylor’s power law ($a = 1.35$, $b = 1.13$, $n = 14$, $r^2 = 0.98$). The $b$ value was significantly greater than one ($t = 3.43$, df = 12, $P <$
Table 1. Binomial sampling table for *P. elmaella* on apples using 20 leaves per tree sample size

<table>
<thead>
<tr>
<th>No. leaves</th>
<th>Proportion of leaves infested &gt;1 mine</th>
<th>Estimated no. mines per leaf</th>
<th>No. leaves</th>
<th>Proportion of leaves infested &gt;1 mine</th>
<th>Estimated no. mines per leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>infested &gt;1 mine</td>
<td></td>
<td></td>
<td>infested &gt;1 mine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.05</td>
<td>0.33</td>
<td>10</td>
<td>0.50</td>
<td>1.74</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>0.51</td>
<td>11</td>
<td>0.55</td>
<td>1.92</td>
</tr>
<tr>
<td>3</td>
<td>0.15</td>
<td>0.67</td>
<td>12</td>
<td>0.60</td>
<td>2.11</td>
</tr>
<tr>
<td>4</td>
<td>0.20</td>
<td>0.81</td>
<td>13</td>
<td>0.65</td>
<td>2.34</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>0.96</td>
<td>14</td>
<td>0.70</td>
<td>2.58</td>
</tr>
<tr>
<td>6</td>
<td>0.30</td>
<td>1.10</td>
<td>15</td>
<td>0.75</td>
<td>2.85</td>
</tr>
<tr>
<td>7</td>
<td>0.35</td>
<td>1.28</td>
<td>16</td>
<td>0.80</td>
<td>3.20</td>
</tr>
<tr>
<td>8</td>
<td>0.40</td>
<td>1.40</td>
<td>17</td>
<td>0.85</td>
<td>3.62</td>
</tr>
<tr>
<td>9</td>
<td>0.45</td>
<td>1.56</td>
<td>18</td>
<td>0.90</td>
<td>4.20</td>
</tr>
</tbody>
</table>

0.01). Equation 9 indicated that 10 trees need to be sampled to accurately predict a threshold of three mines per leaf, and four trees for a two mines per leaf threshold within a one mine confidence interval.

**Binomial Sampling.** Model 1 (equation 5) accurately predicted the mean population level from the proportion of leaves infested (Fig. 1a and b). There were no significant differences between the observed and expected values according to $\chi^2$ for either the large data set used to develop the sampling plan ($\chi^2 = 280.8, 870$ df, $P > 0.99$) or the smaller validation data set ($\chi^2 = 35.9, 245$ df, $P > 0.99$). However, the proportion infested became >0.80 at $\approx 1.7$ mines per leaf, making predictions unstable before the threshold of three mines per leaf used in Utah orchards was approached. This was evident in the validation data set which had a much higher mean number of mines present because of the large infestation in the orchard selected (randomly) from 1987.

The second model binomial sample (equations 6 and 7) also provided accurate predictions of the mean population level from the proportion of leaves infested with greater than one mine (Fig. 2a and b). There were no significant differences between the predicted and observed values for either the large or the validation data set according to $\chi^2$ analysis ($\chi^2 = 328.8, 683$ df, $P > 0.99$; $\chi^2 = 55.2, 179$ df, $P > 0.99$, respectively). Using the second model allowed predictions of the mean population level up to $\approx 3.2$ mines per leaf before $P(>1)$ became >0.80.

Practical use of the sampling plan is determined by the threshold used by the grower. In Utah, the currently used threshold for leafminers is three mines per leaf during the second generation. This requires that 10 trees be sampled per orchard. A total of 20 leaves per tree are removed, scored, and recorded as the number of leaves per tree infested with two or more leafminers and converted to a mean number of mines per tree using Table 1. After all 10 trees were sampled, the orchardwide mean number of mines per leaf was calculated as the average number of mines per leaf from the 10 tree samples.

The sampling plan presented here will provide rapid and reliable estimates of leafminer damage. However, growers should be cautioned that this plan estimates damage which accumulates over the entire season rather than just the number of live larvae. Therefore, once the threshold is exceeded and the grower treats for leafminer, estimates will indicate the seasonal damage already sustained and growers should not base further spraying on the three mines per leaf threshold, but instead on the number of larvae (if any) which survive treatment and the number of parasites present.

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