Enumerative and Binomial Sequential Sampling Plans for the Multicolored Asian Lady Beetle (Coleoptera: Coccinellidae) in Wine Grapes

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ABSTRACT To develop a practical integrated pest management (IPM) system for the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), in wine grapes, we assessed the spatial distribution of *H. axyridis* and developed eight sampling plans to estimate adult density or infestation level in grape clusters. We used 49 data sets collected from commercial vineyards in 2004 and 2005, in Minnesota and Wisconsin. Enumerative plans were developed using two precision levels (0.10 and 0.25); the six binomial plans reflected six unique action thresholds (3, 7, 12, 18, 22, and 31% of cluster samples infested with at least one *H. axyridis*). The spatial distribution of *H. axyridis* in wine grapes was aggregated, independent of cultivar and year, but it was more randomly distributed as mean density declined. The average sample number (ASN) for each sampling plan was determined using resampling software. For research purposes, an enumerative plan with a precision level of 0.10 (SE/X) resulted in a mean ASN of 546 clusters. For IPM applications, the enumerative plan with a precision level of 0.25 resulted in a mean ASN of 180 clusters. In contrast, the binomial plans resulted in much lower ASNs and provided high probabilities of arriving at correct "treat or no-treat" decisions, making these plans more efficient for IPM applications. For a tally threshold of one adult per cluster, the operating characteristic curves for the six action thresholds provided binomial sequential sampling plans with mean ASNs of only 19–26 clusters, and probabilities of making correct decisions between 83 and 96%. The benefits of the binomial sampling plans are discussed within the context of improving IPM programs for wine grapes.

KEY WORDS *Harmonia axyridis*, resampling software, wine grapes, contaminant pest

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), has recently become an economically significant contaminant pest in the wine-making process in the eastern United States. *Harmonia axyridis* adults tend to aggregate on clusters with injured berries just before harvest, and eventually they may be incorporated with the grapes during wine processing (Koch et al. 2004, Pickering et al. 2004). Once disturbed or crushed, *H. axyridis* releases a yellow fluid, via reflex bleeding, that contains alkaloids and alkylmethoxypyrazines that may be used as a defense mechanism or aggregation pheromone (Al Abassi et al. 1998, Dixon 2000). In addition, alkylmethoxypyrazines could be responsible for affecting wine flavor after *H. axyridis* has been crushed along with the grapes (Pickering et al. 2005). Sensory thresholds for several alkylmethoxypyrazines range from 1 to 2 ng/liter in water (Seifert et al. 1970) and from 10 to 26 ng/liter in wine (Boubée et al. 2000).

Known sensory thresholds of alkylmethoxypyrazines released by *H. axyridis* in wine are scarce, and only one study (Pickering et al. 2006a) has determined the sensory threshold for a grape cultivar ('Riesling'), ≈0.2 beetle per cluster. Such a low threshold demonstrates the potential damage of *H. axyridis* to the wine industry. Tainted wine and the unacceptable taste associated with it could lead to economic losses for the wine industry in Minnesota and other states and provinces in the Great Lakes region. In addition, the table grape market also could be affected by *H. axyridis*, primarily under high infestation levels, when the presence of beetles in table grape clusters would become a nuisance.

Even though remediation of tainted wine, by adding oak chips, activated charcoal, and deodorized oak has decreased *H. axyridis*-related taint, it has not completely removed the taint from contaminated wine (Pickering et al. 2006b). Therefore, the use of control measures such as insecticides to manage *H. axyridis*, before it can become a wine contaminant, is essential for reducing the economic impact of this pest in the wine industry. However, insecticide application without sampling protocols may result in unnecessary increases in production costs and in environmental con-

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tamination. Within the context of pest management (e.g., *H. axyridis* in wine grapes), practical sampling plans are required for making correct control decisions (e.g., Binns et al. 2000). Management decisions should be made based on an assessment of the pest density or infestation level (Binns and Nyrop 1992). Sequential sampling has been used widely for decision making, because the approach can significantly reduce sampling effort and retain a desired level of precision (Binns 1994, Hutchison 1994, Hodgson et al. 2004). Enumerative sequential sampling is primarily used for estimating population density for research purposes (Green 1970, Hutchison 1994), but it also can be used for decision making (Hodgson et al. 2004). Binomial (i.e., presence/absence) sequential sampling is most often used for integrated pest management (IPM) decision making (Binns 1994), either directly, or by estimating mean density from the density–binomial relationship (Nyrop et al. 1989, Jones 1994).

The decision to treat wine grapes with an insecticide for control *H. axyridis* is currently being made on a calendar basis without a formal sampling procedure to determine whether the population level is causing economic damage. The initiation of a spray program is determined on the preharvest interval of labeled insecticides, and the anticipated harvest date. Therefore, the development and validation of practical and efficient decision-making tools are essential first steps for an IPM for *H. axyridis* in wine grapes.

In this article, we present eight sequential sampling plans, and we evaluate their performance via computer simulation. Two of the plans were developed for enumerative sampling with different fixed precision levels, 0.10 for research purposes and 0.25 for IPM applications (Southwood and Henderson 2000). Six of the plans were developed for binomial sampling (presence/absence of *H. axyridis* in clusters). Because physical characteristics, chemical characteristics, or both of various wine grapes may yield different action thresholds, each binomial plan refers to a specific action threshold (i.e., 3, 7, 12, 18, 22, and 31% of cluster samples infested with at least one *H. axyridis* adult). The development and validation of sampling plans were done using the resampling software Resampling for Validation of Sample Plans (Naranjo and Hutchison 1997). Within the context of binomial sampling for IPM applications, we also determined the probability of making correct treat or no-treat decisions for several action thresholds.

Materials and Methods

Commercial vineyards of the wine grapes 'Frontenac', 'Marechal Foch', and 'Leon Millot' were sampled in 2004 and 2005. Vineyards were located in Hastings, MN, in 2004 and in Hastings, Stillwater, Afton, and Red Wing, MN, and Somerset in Wisconsin in 2005. In 2004, 23 data sets were collected from 11 August to 21 September, and in 2005, 26 data sets were collected from 27 July to 16 September. Vineyards ranged from 0.5 to 5.0 ha. On each sample date, 40–340 randomly selected clusters were sampled using visual whole-cluster inspection to estimate *H. axyridis* densities. We used nondestructive cluster sampling by carefully inspecting each cluster in the vines. *H. axyridis* adult densities on each cluster were recorded; ≈13 s was required to estimate *H. axyridis* density per cluster. *H. axyridis* adults were identified using a diagnostic guide (Schellhorn 2003), and voucher specimens were deposited in the Insect Museum in the Department of Entomology (University of Minnesota). Mean densities ranged from 0.004 to 2.125 *H. axyridis* per cluster. In 2004, a uniform grid sampling pattern was used with 4–34 sample points, depending on vineyard size. Ten clusters (sample units) were visually inspected per sample point. A uniform grid sampling pattern was used to determine whether *H. axyridis* population would concentrate on the edges of the vineyards. However, no edge-effect was found, and, in 2005, a random sampling pattern was used where 40–220 sample units were randomly selected from points across whole vineyard.

Enumerative Sampling. Development and validation of enumerative sampling plans require two steps. First, we assessed the spatial distribution of the insect pest using Taylor's power law (Taylor 1961). Based on previous studies (Pena and Duncan 1992, Cho et al. 1995), Taylor's power law adequately describes the spatial distribution of insect populations, because *H. axyridis* occurs at low densities in vineyards (Fig. 1). Second, we developed and validated the stop lines using field-collected data sets and the Resampling for Validation of Sample Plans software. The software uses Green's plan (Green 1970), which requires three parameters: a and b values from Taylor's power law, and a desired precision level.

We randomly selected 35 of 49 data sets to calculate a and b values for Taylor's power law ($s^2 = am$), which describes the mean (m) to variance (s^2) ratio and the spatial distribution of a species (Taylor 1961). The a and b values of Taylor's power law were determined for each cultivar (Frontenac, Marechal Foch, and Leon Millot), year (2004 and 2005), and for two range of mean densities of *H. axyridis* per cluster (i.e., 0.004–0.045 and 0.05–2.125). The a and b values were calculated using linear regression analysis (PROC REG, SAS Institute, 2003) of the log of mean and the log of variance (Taylor 1961). Student t-tests (PROC REG, SAS Institute, 2003) were used to determine whether the slopes (b values of Taylor's power law) of the regression lines were significantly >1.0. Homogeneity of regression slopes and equality of intercepts over different cultivar, year, and density were tested with analysis of covariance (ANCOVA) (PROC GLM, SAS Institute, 2003) (Sokal and Rohlf 1995). For each ANCOVA, cultivar, year, or density was the covariate and mean number of *H. axyridis* was the main effect. If the interaction between the main effect and each covariate was not significantly different ($P > 0.05$), the slopes were homogenous. If the levels of each covariate were not significantly different ($P > 0.05$), the intercepts were equal. The a and b parameters of Taylor's power law from the 35 randomly selected data sets were used to develop the two enumerative sam-

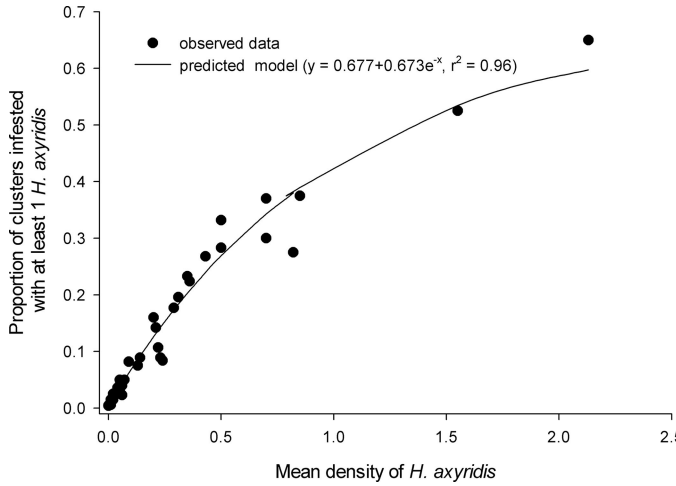

Fig. 1. Nonlinear relationship between the proportion of clusters infested with at least one *H. axyridis* per cluster (tally threshold, one *H. axyridis* per cluster) and the mean number of *H. axyridis* per cluster.

pling plans for estimating the total number of *H. axyridis* per cluster (Green 1970). The remaining 14 data sets, representing a range of 0.004–2.125 *H. axyridis* per cluster, were used in validating the sampling plans with the Resampling for Validation of Sample Plans software.

The sampling stop line for Green's plan was calculated by the following formula:

$$T_n \geq (an^{1-b}/SE/\bar{X}^2)^{1/(2-b)},$$

where T_n is the cumulative number of individuals found, n is the total number of sample units, $SE/\bar{X}$ is the desired precision level, and a and b are the Taylor's power law parameters. Independent clusters were sampled sequentially and the numbers of *H. axyridis* in each cluster were recorded (Green 1970). The cumulative number of *H. axyridis* in clusters was plotted against the cumulative number of clusters with reference to the stop line. Sampling stopped when the stop line was crossed. The fixed precision levels used to estimate the stop lines were 0.10, commonly used for ecological purposes, and 0.25, normally used for pest management applications (Southwood and Henderson 2000). Resampling analysis for mean actual density, mean sampling number, and mean actual precision was based on 500 sampling runs. The average sample number from the validation data sets was selected as the recommended sample size for the density range tested.

Binomial Sampling. Binomial sampling plans for *H. axyridis* in wine grapes were developed and validated in three steps. First, the proportion of clusters infested with at least one *H. axyridis* was related to mean density by using all data sets (Fig. 1). This relationship was used to transform the proposed mean density action thresholds (i.e., 0.05, 0.1, 0.2, 0.3, 0.4, and 0.6 *H. axyridis* per cluster) to proportion infested action thresholds (i.e., 3, 7, 12, 18, 22, and 31% of cluster samples infested). Second, the operating characteristic function was calculated for each action threshold. The operating characteristic function estimated the probability of not treating an insect population relative to the insect density or the proportion of clusters infested (Binns et al. 2000). Third, the stop lines were developed for each action threshold using the sequential probability ratio test (Wald 1947).

Binomial sampling plans were developed and validated using all data sets to create decision stop lines for the Wald (1947) sequential probability ratio test with Resampling for Validation of Sample Plans software (Naranjo and Hutchison 1997). Stop lines for a sequential probability ratio test require parameters for θ_1 and θ_2, the lower and upper boundaries, respectively, for the decision action threshold, and α (type I) and β (type II) error rates associated with the decision boundaries (Jones 1994). A type I error would be to treat when actual pest density was below the action threshold. A type II error would be to avoid treating when actual pest density was above the action threshold. All parameters were held constant for the tally threshold of one *H. axyridis* per cluster (i.e., clusters with ≥1 *H. axyridis* were considered infested), and the six selected action thresholds.

The tally threshold of one *H. axyridis* per cluster was based on the sensory thresholds, suggested by grape grower associations and wineries, which were lower than one *H. axyridis* per cluster (e.g., Pickering et al. 2006a). Similarly, the six action threshold also represented a range of sensory thresholds suggested by the grape grower associations and wineries. To date, the only sensory threshold is for the white grape Riesling (≈0.2 *H. axyridis* per cluster or 12% of cluster samples infested based on the population densities of our data sets; Fig. 1) (Pickering et al. 2006a). Because each grape cultivar has unique physical and chemical characteristics, including natural levels of alkylmethoxypyrazines present in 'Cabernet sauvignon' and 'Sauvignon blanc' (Allen et al. 1994, Sala et al. 2002), each

cultivar may need to have its own sensory threshold. In addition, wine processing for white and red cultivars also could affect the sensory threshold (Pickering et al. 2006a). Therefore, the action thresholds chosen here were based on possible sensory thresholds for each cultivar.

The upper and lower boundaries of the action threshold, θ_1 and θ_2 (=0.10 above and below the action threshold, respectively), and type I ($\alpha = 0.10$) and type II ($\beta = 0.01$) error rates were used to develop the sampling plans. Normally binomial plans set α and β at 0.10 (Jones 1994, Naranjo and Hutchison 1997, Burkness et al. 1999, Hodgson et al. 2004). However, in this study, we set the $\beta = 0.01$, because the market value of wine grapes may be 100 times higher than control costs, and because without control of *H. axyridis*, the result is complete loss of the wine. Decreasing β results in wider stop boundaries, higher average sample number values, and steeper operating characteristic function (Binns et al. 2000). The reduction of β is a compromise between cost and reliability, because it increases the average sample number, and, consequently, the costs of sampling, but it also increases its reliability (Jones 1994).

Stop lines for each action threshold were calculated using Wald's plan and were defined as follows:

$$T_{n(t)} \geq Rx + Q \text{ and } T_{n(t)} \leq Rx - S,$$

where $T_{n(t)}$ is the cumulative number of samples infested with at least t insects, and Q, R, and S are functions of α and β. After resampling analysis, the average proportion infested, average sample number, and the operating characteristic function were calculated and summarized based on 500 iterative sampling runs. Resampling outputs also provide actual α and β values for each sample comparison.

Precision of Binomial Sampling Plans. Sequential plans for decision making classification should be based on precision and efficiency. Binns and Nyrop (1992) described the use of the operating characteristic function to determine precision and the average sample number function to determine efficiency. The operating characteristic function is the probability of making a treatment decision (whether correctly or not), and the average sample number function is the expected sample size for making a decision, but it does not calculate the costs for the sampling procedure. Sampling plans have to be precise, with a high probability of making correct decisions and sampling must be relatively inexpensive in terms of time to take the sample and to process any data collected. In this study, a four-cell probability matrix (e.g., Calvin et al. 1986, Burkness et al. 1999), which includes the probability of making a correct decision, was used to assess the precision of sampling plans. In addition, relative net precision for enumerative counts, and benefit–cost ratios for binomial counts were used to measure the efficiency of sampling plans (Burkness et al. 1999, Hodgson et al. 2004). Both relative net precision and benefit–cost ratios include costs and precision of sampling plans in their determination.

Precision is defined as how close an estimated mean number (m) from a sampling procedure in a selected population is to the expected number (E) in the same population, and can be quantified by $E(m - E(m))^2$, which increases as estimates become more variable (Binns et al. 2000). The four-cell matrix characterize the probability of making a correct decision (treat or not treat) based on the comparison of the estimated proportion infested from the simulation to the observed proportion infested at each action threshold. Therefore, precision of binomial plans can be defined as how close an estimated observation (i.e., estimated proportion infested from the simulation) from a sampling procedure in a selected population is to the expected observation (i.e., observed proportion infested at each action threshold) in the same population. In this case, as the probability of making a correct decision decreases, the estimates from the sampling plan become more variable.

The four-cell probability matrix was calculated for each action threshold. Estimated proportion infested and operating characteristic values from the simulation were used to determine the probability of making a correct decision (i.e., treat and not treat). The decision to treat or not was determined by comparing the estimated proportion infested from the simulation to the proportion infested at each action threshold (Burkness et al. 1999). That is, if the proportion of infested clusters is higher than the proportion of infested clusters at action threshold, the decision is to treat. However, if the proportion of infested clusters is lower than the proportion of infested clusters at action threshold, the decision is not to treat (Burkness et al. 1999). The four-cell probability matrix-included cells A (correct decision to treat), D (correct decision not to treat), B (incorrect decision to treat), and C (incorrect decision not to treat). An appropriate decision for each data set is fixed by the magnitude of infestation and has to be correct or incorrect in the matrix, where A + B = 1 or C + D = 1 (Burkness et al. 1999, Hodgson et al. 2004). Therefore, if the infestation is high enough to determine a treatment, the probability of A = 1 − OC (operating characteristic) and the probability of B = OC. However, if the infestation is low enough to determine a treatment, the probability of C = 1 − OC and the probability of D = OC. The probability of making a correct decision was summarized for all data sets at each action threshold with

$$1 = \sum p_i (A_i + D_i) + \sum p_i (B_i + C_i),$$

where p_i is the proportion of n data sets represented by data set i, A, D, B, and C were defined above.

Efficiency of Enumerative and Binomial Sampling Plans. Relative net precision was used to compare the two enumerative sampling plans, because it gives equal consideration to precision and sampling time (Pedigo et al. 1972). Efficiency of sampling plan increases with higher relative net precision, which is calculated a follows:

$$\text{relative net precision} = (1/(RV \times c)) \times 100,$$

Table 1. Statistics of Taylor's power law parameters (*a* and *b*), mean range and mean densities for variety (Frontenac, Marechal Foch, and Leon Millot), year (2004 and 2005), two levels of mean density of *H. axyridis* per cluster (lower and higher), and the 35 randomly selected data sets for Green's plan

Source	*n*	$a \pm$ SEM	$b \pm$ SEM	r^2	H_0: $b > 1$ (t, P)	Mean range	Mean density ± SEM
Variety							
Marechal Foch	18	4.57 ± 0.11	1.29 ± 0.07	0.95	4.16, 0.0007	0.0045–0.7037	0.1299 ± 0.0435
Frontenac	18	1.95 ± 0.04	1.14 ± 0.02	0.99	5.76, <0.0001	0.0041–0.5	0.1065 ± 0.0374
Leon Millot	13	3.16 ± 0.04	1.26 ± 0.04	0.99	6.70, <0.0001	0.0083–2.125	0.5645 ± 0.1805
Yr							
2004	23	3.31 ± 0.06	1.22 ± 0.06	0.95	4.36, 0.0024	0.0041–2.125	0.4359 ± 0.1085
2005	26	2.29 ± 0.04	1.17 ± 0.03	0.99	6.27, <0.0001	0.0045–0.5	0.0602 ± 0.0224
Mean density							
Lower density	22	1.38 ± 0.06	1.06 ± 0.03	0.98	2.07, 0.0516	0.0041–0.0454	0.0131 ± 0.0023
Higher density	27	3.09 ± 0.07	1.21 ± 0.09	0.87	2.31, 0.0291	0.05–2.125	0.4187 ± 0.0925
Green's plan							
Random selection	35	3.28 ± 0.06	1.24 ± 0.04	0.96	5.91, <0.0001	0.0045–1.55	0.2015 ± 0.0552

where RV is the relative variation $(SE/\bar{X}) \times 100$ (Southwood and Henderson 2000), and c is the total cost related to collecting the selected sample, usually measured in person-hours.

Benefit–cost ratio was used to compare the six binomial sampling plans by using the proportion of total correct decisions from the four-cell matrix, and the cost of obtaining infestation estimates (Burkness et al. 1999, Hodgson et al. 2004). The benefit–cost ratio was calculated as follows:

$$\text{benefit–cost ratio} = [\Sigma P_c/(n \times c)] \times 100,$$

where P_c is the sum of proportional correct decisions, n is the average sample size required to make a decision, and c is the cost of collecting the sample (Hodgson et al. 2004).

Results

Enumerative Sampling. Regression analyses of the log-mean and log-variance showed positive linear correlations for cultivar, year, and density of *H. axyridis* per cluster, with coefficients of determination (r^2) ranging from 0.95 to 0.99, except for the higher density of *H. axyridis* (0.87) (Table 1). The strong relationship shows that Taylor's power law fits the data well. All b values from Taylor's power law were statistically >1 ($P < 0.05$), except at lower densities, where P values were slightly >0.05 ($P = 0.0516$) (Table 1). These results suggest an aggregated distribution for *H. axyridis* in the vineyards, except at the lower densities where *H. axyridis* populations reflect a more random distribution (b of Taylor's power law = 1). In addition, the 35 randomly selected data sets for Green's plan also showed positive linear correlations between log-mean and log-variance ($r^2 = 0.96$), and a b value significantly >1 ($P < 0.0001$) (Table 1).

ANCOVA showed that regression slopes were equal across cutlivars ($F = 2.63$; df = 2, 43; $P = 0.084$), years ($F = 0.49$; df = 1, 45; $P = 0.488$), and densities ($F = 0.25$; df = 1, 45; $P = 0.620$). However, intercepts were statistically different where data sets were grouped by cultivars ($F = 4.04$; df = 2, 45; $P = 0.024$) and by densities ($F = 5.91$; df = 1, 46; $P = 0.019$), but they were equal in 2004 and 2005 ($F = 2.78$; df = 1, 46; $P = 0.102$). These results indicate that *H. axyridis* adults had a similar mean-to-variance relationship in 2004 and 2005, and similar slopes among the Marechal Foch, Frontenac, and Leon Millot, and between high and low densities. The equal regression slopes support the combination of all data sets for determination of *H. axyridis* spatial distribution and, consequently, the enumerative sampling plan.

Validation of Green's plan was not possible with data sets that had mean densities lower than 0.2 or 0.05 *H. axyridis* per cluster for a desired precision level of 0.10 and 0.25, respectively. Therefore, only six (0.10 precision level) and nine (0.25 precision level) independent data sets were used to validate enumerative plans using Resampling for Validation of Sample Plans software (Fig. 2). Independent of the desired precision level, sample size requirements decreased as mean density increased (Fig. 2). Modification in the initial fixed precision level can result in adjustments to the final actual precision level and sample size obtained (Hutchison et al. 1988). We decreased the initial precision to 0.11 and 0.27 to obtain the actual, desired precision levels of 0.10 and 0.25, and average sample numbers of 546 and 180 clusters, respectively. The average maximum and minimum sample sizes were 689 and 187 clusters at the 0.10 precision level, and 315 and 93 clusters at the 0.25 precision level. Actual average maximum and minimum precision levels were 0.115 and 0.085 at the desired 0.10 precision level, and 0.32 and 0.185 at the desired 0.25 precision level.

Binomial Sampling. The operating characteristic function curves showed that the probability of not treating at the action threshold for all six binomial plans was below 0.50, suggesting that these sampling plans are conservative (Fig. 3A–F). That is, treatment was more likely to occur than no-treatment at the action threshold. The operating characteristic function represents the probability of not treating, and when the actual pest density is at the action threshold, the operating characteristic is 0.50; that is, there is 50% chance of treating or not treating (Binns et al. 2000).

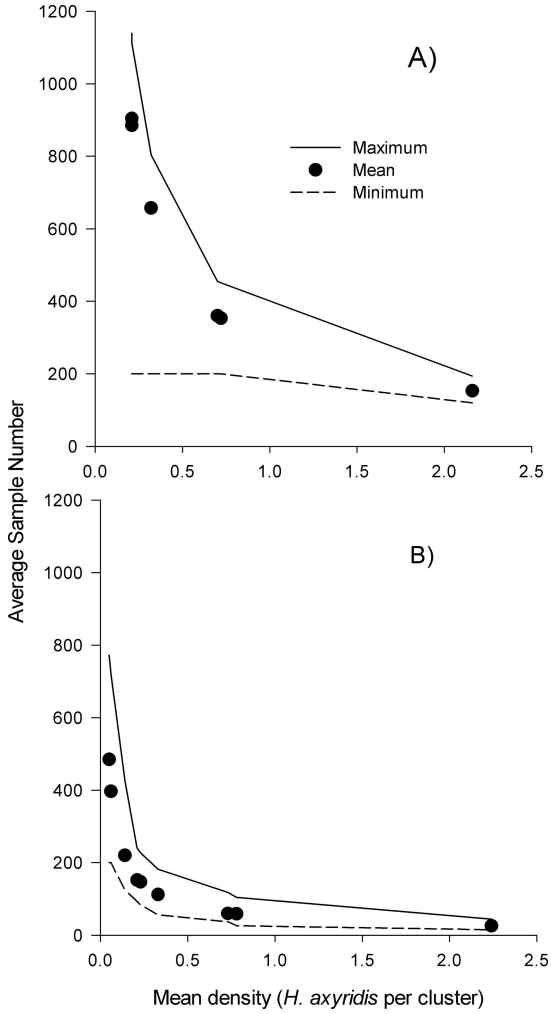

Fig. 2. Summary of resampling validation analysis showing average sample number for Green's sequential plan over a range of *H. axyridis* mean densities. The average sample number is the mean sample size for the density range tested, and it changes with the density. (A) Green's plan with preset precision of 0.11 resulting in an actual precision of 0.10. (B) Green's plan with preset precision of 0.27 yielding an actual precision of 0.25.

However, because wine grapes are a valuable crop, we preset the type II error (i.e., probability of not treating when population density is above the action threshold) to 0.01, resulting in a lower operating characteristic (<0.50) at the action threshold, and thus in a conservative plan. In addition to the crop value, grower expectations (risk-taker or risk-adverse), and control method options (e.g., chemical or biological) also may influence the relationship between operating characteristic function and action threshold (Binns et al. 2000).

The stop lines for the six binomial plans show that independent of the chosen action threshold, the average sample number was lower than in the two enumerative plans (Fig. 4A–F). First, 19–25 (depending on the action threshold) clusters are sampled and the presence or absence of *H. axyridis* is recorded. Second, the proportion of infested clusters is calculated from the sampled clusters and compared with the stop line graphs (Fig. 4). Third, a management decision is made if the proportion is in the "treat" or "do not treat" area. However, if the proportion is in the "continue sampling" area, more samples have to be taken before a treatment decision is made. Because the sampling plan is sequential, the proportion can be in the "continue sampling" area perpetually. Therefore, to make the binomial plan a practical tool for IPM, we recommend not examining >50 clusters to arrive at a decision. If, after 50 clusters, a decision cannot be made, the vineyard must be resampled at a later time. The period for resampling the same vineyard should be based on the projected time of harvest and on the preharvest interval of available insecticides.

In each graph of Fig. 4, the mean average sample number was calculated based on 500 iterative sampling runs after resampling analysis. Therefore, the average sample number shown for each action threshold is the mean sample size for the 500 sampling runs. Actual sample size for the binomial sampling plans, for any given sample date, will depend on the *H. axyridis* infestation level and the action threshold. However, given the infestation levels found in the vineyards that form the basis for developing these sampling plans, the mean average sample number ranged from 19 and 25 clusters per sample.

The probability of correctly deciding to treat or not treat ranged from 82 to 96% (Table 2). In addition, the probability of incorrectly deciding to not treat when population density was above the action threshold (type II error) was 1% in five of the six sampling plans (Table 2). That is, a grower may make a wrong decision of not spraying when they should, in only one out of every 100 decisions. Therefore, even though the binomial sequential sampling plans for *H. axyridis* in wine grapes had a smaller sample size (19–25 clusters), compared with the enumerative sampling plans (546 and 180 clusters), the binomial sampling plans maintained a high probability of making correct treatment decisions.

Efficiency of Enumerative and Binomial Sampling Plans. Efficiency of the enumerative sampling plans was calculated based on a whole cluster sample unit. The average sample time per cluster per person (13.1 s or 0.003628 h) included the walk time between samples. The sample time of 13.1 s also was used to calculate the efficiency of binomial sampling plans. The time to examine one cluster by using binomial counts was similar to that to sample one cluster by using enumerative counts, for three reasons. First, a grape cluster is a relatively small sample unit. Second, *H. axyridis* is a conspicuous insect in the cluster because of its bright red and black colors and its size. Third, the mean density is normally lower than one *H. axyridis* per cluster; only two of the 49 data sets used in this research had mean densities higher than one *H. axyridis* per cluster.

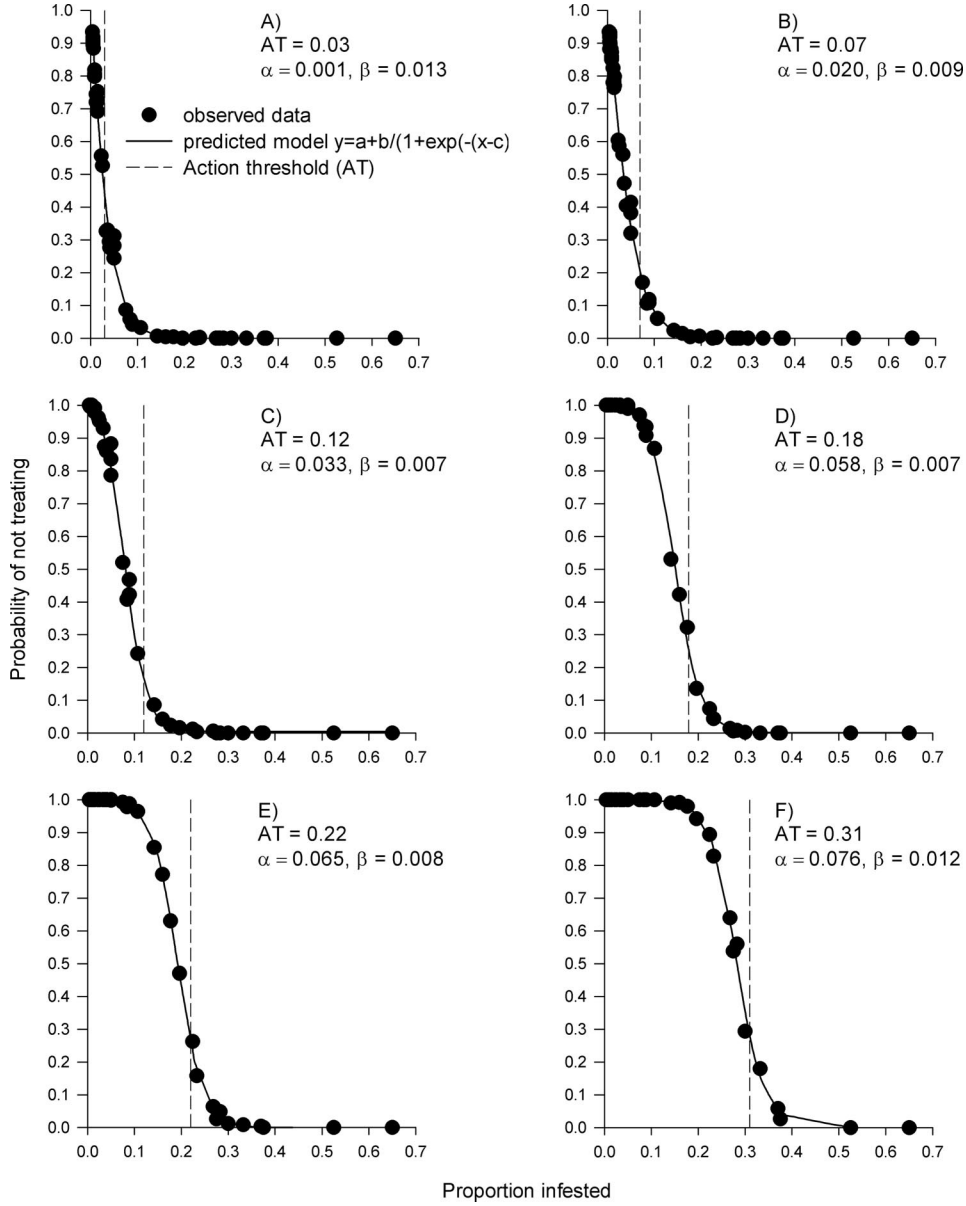

Fig. 3. Operating characteristic (OC) function for binomial sequential sampling plans for *H. axyridis* in wine grapes. The OC was plotted against the observed proportion infested (tally threshold, one *H. axyridis* per cluster) obtained from resampling software; action thresholds and actual α and β values are shown, based on preset values of $\alpha = 0.10$ and $\beta = 0.01$.

The relative net precision for the 0.10 fixed precision enumerative sampling plan was lower than the 0.25 fixed precision level (Table 3). Therefore, the efficiency of the 0.25 fixed precision level sampling plan used for IPM purposes was more efficient than the 0.10 fixed precision sampling plan for research applications.

Discussion

In this study, we determined that the spatial distribution of *H. axyridis* adults in wine grapes was aggregated, independent of the cultivar and year. We also verified that spatial pattern of *H. axyridis* became more randomly distributed as the mean density decreased. In addition, *H. axyridis* had a similar mean-to-variance relationship across cultivars, independent of mean densities.

The distribution of *H. axyridis* has been shown to be aggregated or random, depending on life stage or cropping system (Ren et al. 2000, Park and Obrycki 2004, Koch et al. 2006). Aggregation has been reported more often in the larval stages (Ren et al. 2000, Koch et al. 2006) and at high densities (Park and Obrycki

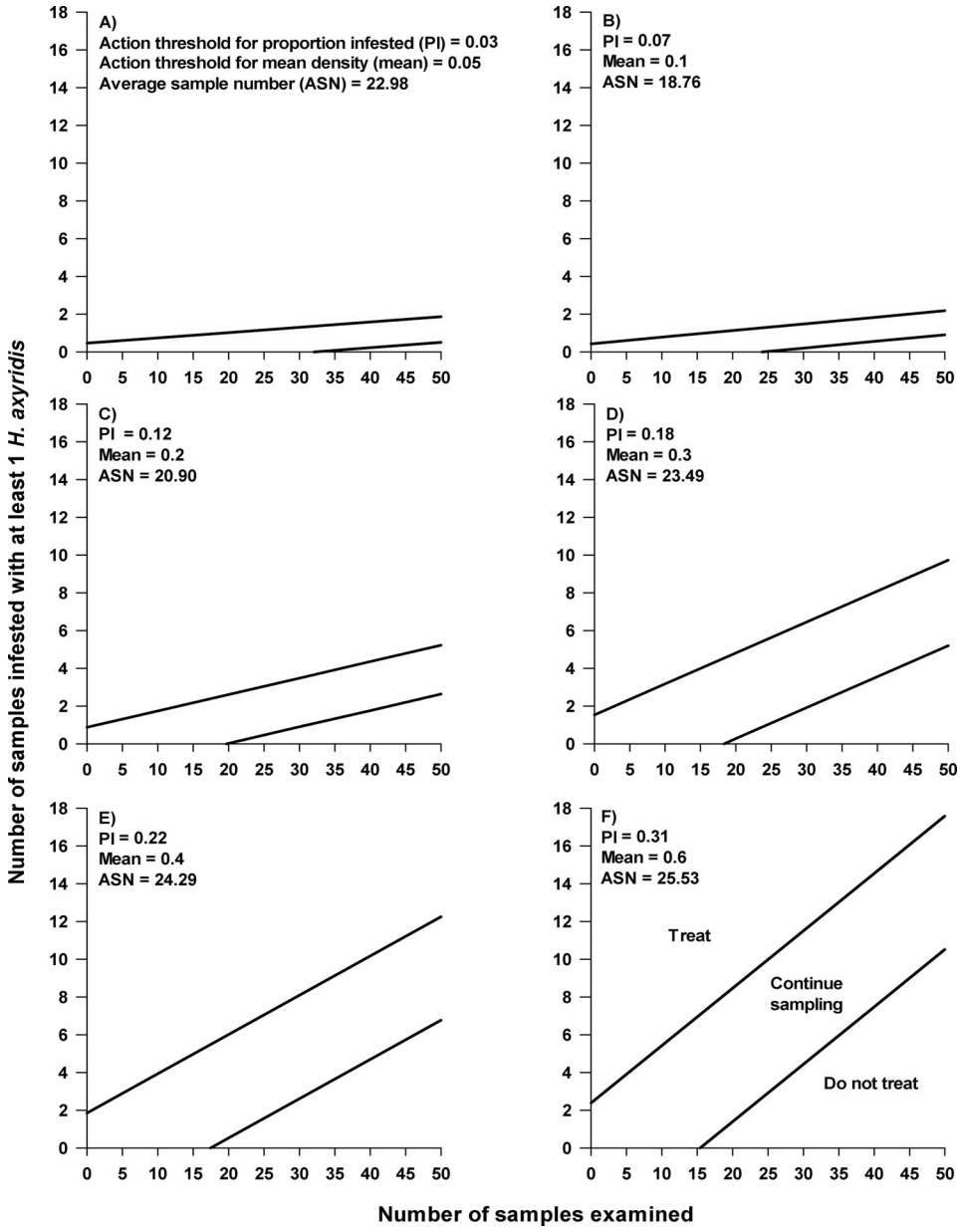

Fig. 4. Decision stop lines for the binomial sequential plans for *H. axyridis* adults based on resampling analysis, six action thresholds, and a tally threshold of one (≥1 *H. axyridis* adults per cluster to be considered infested). ASN was calculated based on 500 iterative sampling runs after resampling analysis. Therefore, the average sample number shown for each action threshold is the mean sample size for the 500 sampling runs. Actual sample size for the binomial sampling plans, for any given sample date, will depend on the *H. axyridis* infestation level and the action threshold.

2004); random spatial pattern is more common in the adult stage (Koch et al. 2006) and at low densities (Park and Obrycki 2004). Aggregated distributions of other coccinellids also have been reported (e.g., Musser et al. 2004), primarily because their prey exhibit an aggregated distribution (Yasuda and Ishikawa 1999, Dixon, 2000). However, in wine grapes, *H. axyridis* adults feed on previously injured berries; consequently, their distribution follows the distribution of injured berries in the vineyards. Therefore, comparisons between the spatial distribution of *H. axyridis* in wine grapes and the distribution of *H. axyridis*, as well as other predatory coccinellids, in crops where lady beetles are searching for prey, have limited ecological relevance.

We developed two enumerative sampling plans for *H. axyridis* in wine grapes, including research (0.10 precision) and pest management (0.25 precision) ap-

Table 2. Probabilities of correct and incorrect treatment decisions for various action thresholds for *H. axyridis* in wine grapes

Mean *H. axyridis*[a]	AT[b]	α[c]	β[c]	Correct decision			Incorrect decision			Sample size
				A[d]	D[e]	A + D[f]	B[g]	C[h]	B + C[i]	
0.05	0.03	0.001	0.013	0.503	0.353	0.856	0.048	0.096	0.144	22.98
0.10	0.07	0.020	0.009	0.396	0.433	0.829	0.012	0.159	0.171	18.76
0.20	0.12	0.033	0.007	0.302	0.610	0.912	0.004	0.084	0.088	20.90
0.30	0.18	0.058	0.007	0.239	0.711	0.950	0.006	0.044	0.050	23.49
0.40	0.22	0.065	0.008	0.213	0.748	0.961	0.012	0.028	0.040	24.29
0.60	0.31	0.076	0.012	0.111	0.844	0.955	0.011	0.033	0.044	25.53

[a] Mean densities that originated action threshold values based on the proportion of clusters infested and mean relationship curves (see Fig. 3)

[b] Action threshold, six levels of infestation that represent a potential range of *H. axyridis* action thresholds.

[c] Type I error (α) is defined as making a treat decision when actual pest density is below the action threshold. Type II error (β) is defined as making a no-treat decision when actual pest density is above the action threshold. Type I error value was preset at 0.10, and type II error value was preset at 0.01 for resampling simulations. Actual error values were estimated from the fitted curves in Fig. 3.

[d] A, probability of both the estimated proportion of clusters infested and the true population being above their respective action threshold resulting in a correct treat decision.

[e] D, probability of both the estimated proportion of clusters infested and the true population being below their respective action threshold resulting in a correct no-treat decision.

[f] A + D, probability of making a correct treat or no-treat decision.

[g] B, probability of both the estimated proportion of clusters infested being below the proportion infested action threshold, and the true population is above the density action threshold resulting in an incorrect no-treat decision.

[h] C, probability of both the estimated proportion of clusters infested being above the proportion infested action threshold, and the true population is below the density action threshold resulting in an incorrect treat decision.

[i] B + C, probability of making an incorrect treat or no-treat decision.

plications. However, for management purposes the binomial sampling plans showed the highest efficiency, independent of the action threshold. Growers could make a decision to spray or not spray in ≈5 min by using the binomial sampling plans (Table 3). In addition to the short sampling time, the binomial sampling plans also offered a high probability of making a correct decision. The combination of short sample time and the high probability of making a correct decision (higher than 83%) resulted in a high benefit–cost ratio for all binomial sampling plans. Therefore, for IPM decisions, a binomial sequential sampling plan is the most efficient option to characterize *H. axyridis* populations in wine grapes. Wine grape growers should start to sample 2–3 wk before harvest and should increase sampling frequency in the last week before the harvest. The actual time for sampling during the last week should be based on the postharvest interval (PHI) of insecticides that are labeled for wine grapes. For example, for an insecticide with 3-d PHI, sampling should be done 5 or 6 d before harvest, to also provide ample time implement control.

Research is in progress to determine a sensory-based threshold, from which a final action threshold will be established, for Frontenac. Action thresholds are expected to be in the range of 0.05–0.60 *H. axyridis* per cluster, and, potentially the binomial sampling plan for Frontenac, as well as for other cultivars, will be one of the sampling plans presented here (T.L.G., unpublished data). However, given the robust nature of the

Table 3. Comparison of efficiency of the enumerative sampling plans by using relative net precision, and of the binomial sampling plans by using benefit cost ratio for *H. axyridis*

Sampling plan	Avg sample no. (n)[a]	Avg sample time (h)[b]	Total sample time (h)[c]	RNP[d]	BCR[e]
Enumerative					
Precision of 0.10 (RV = 10)	546	0.003628	1.981 (118.86 min)	5.05	
Precision of 0.25 (RV = 25)	180	0.003628	0.653 (39.18 min)	6.12	
Binomial					
AT = 0.03, ΣP_c = 0.856	23	0.003628	0.083 (4.98 min)		1025.84
AT = 0.07, ΣP_c = 0.829	19	0.003628	0.069 (4.14 min)		1202.63
AT = 0.12, ΣP_c = 0.912	21	0.003628	0.076 (4.56 min)		1197.04
AT = 0.18, ΣP_c = 0.950	24	0.003628	0.087 (5.22 min)		1091.05
AT = 0.22, ΣP_c = 0.961	24	0.003628	0.087 (5.22 min)		1103.68
AT = 0.31, ΣP_c = 0.955	26	0.003628	0.094 (5.64 min)		1012.42

[a] The average sample number was calculated based on 500 iterative sampling runs after resampling analysis. Therefore, the average sample number shown for each action threshold is the mean sample size for the 500 sampling runs. Actual sample size for the binomial sampling plans, for any given sample date, will depend on the *H. axyridis* infestation level and the action threshold.

[b] Time per cluster per person + walk time between samples in hours.

[c] Time per person in hours include the sample time for sampling the average number of clusters and the walk time between cluster samples.

[d] Relative net precision = $(1/(RV \times c)) \times 100$, where RV is the relative variation ($SE/\bar{X}$), and c is the total cost related to collecting the selected sample size, usually measured in person-hours.

[e] Benefit–cost ratio = $[\Sigma P_c/(n \times c)] \times 100$, where P_c is the sum of proportional correct decisions, n is the average number of samples required to make a decision, and c is the cost (time) of collecting the sample.

binomial plans, using a wide range of action thresholds shown in Fig. 3, we anticipate that the final sampling plan will have similar precision and efficiency.

In addition to the sensory threshold, we also are conducting research to determine whether only the presence of *H. axyridis* in the clusters for 2–3 wk before harvest may be sufficient to taint the wine through releases of alkylmethoxypyrazines on the berries. Presently, for the management of *H. axyridis*, we are assuming that this insect does not release alkylmethoxypyrazines on the berries, at least not enough to contaminate the wine, until they are disturbed or crushed during harvest. Based on this assumption, growers should make management decisions based on the density or infestation level that they find on each sample date. However, although *H. axyridis* is in the clusters, if the beetles release enough alkylmethoxypyrazines on the berries to taint the wine, management decisions should be made based on the cumulative number of *H. axyridis* in the clusters, and not only on the infestation level for a particular sample date.

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