Development and Validation of Node-Based Sample Units for Estimating Soybean Aphid (Hemiptera: Aphididae) Densities in Field Cage Experiments

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ABSTRACT The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is currently the most important insect threat to soybean, *Glycine max* (L.) Merr., production in the North Central United States. Field cage studies are a key tool in investigating the potential of natural enemies and host plant resistance to control this pest. However, a major constraint in the use of cage studies is the limited number of treatments and replicates that can be used as aphid densities frequently become so large as to limit the number of experimental units that can be quantified. One way to overcome this limitation is to develop methods that estimate whole-plant aphid densities based on a reduced sampling plan. Here, we extend an existing method, node-sampling, used for estimating aphid populations in open field conditions and apply it to caged populations. We show that parameters calculated under open field conditions are inappropriate to estimate caged populations. In contrast, using four independent data sets of caged populations and a cross-validation technique, we demonstrate that a three-node sampling unit and a weighted formula provide accurate and robust estimates of whole-plant aphid density. This method reduced the number of aphids counted per plant by and average of 60%, with greater reductions at higher aphid densities. We further demonstrate that nearly identical statistical results were obtained when whole-plant or node-sampling estimates were used in the analysis of two case studies. The reduced sample unit method developed here saves time without sacrificing efficiency so that more plants, replications, or studies can be conducted that will lead to improved soybean aphid management.

KEY WORDS *Aphis glycines*, sampling, field experiments, predation, host plant resistance

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an invasive species that currently represents the major insect threat to soybean, *Glycine max* (L.) Merr., production in the North Central United States and Canada (Ragsdale et al. 2004, 2007; Venette and Ragsdale 2004; Landis et al. 2008). Although it can vector several plant viruses (Davis et al. 2005), most of the yield losses attributed to soybean aphid are caused by feeding injury (Ragsdale et al. 2004, 2007). Recently, Ragsdale et al. (2007) developed an economic injury level and an economic threshold for soybean aphid that is based on the number of aphids per plant in open field plots. Similarly, available sampling plans for soybean aphid are based on whole-plant counts as their sample unit (Hodgson et al. 2004, Onstad et al. 2005). However, estimating whole-plant aphid densities is a time consuming process, especially with high aphid infestation (McCornack et al. 2008).

Current management strategies for soybean aphid are almost exclusively based on insecticide applications (Myers et al. 2005, Ragsdale et al. 2007). Alternative approaches to reduce the dependence on chemical controls involve the development of soybean varieties resistant to soybean aphid (Hesler and Dashiell 2007) and the inclusion of natural enemies into modified economic thresholds (Rutledge et al. 2004, Ragsdale et al. 2007). Field experiments designed to tease apart the different factors that limit aphid growth, including predation and host plant resistance, typically include caged control treatments that ideally allow for unlimited aphid population growth. Under those conditions, soybean aphid populations can grow exponentially (Costamagna et al. 2007b, Matis et al. 2009) and easily reach densities exceeding 10,000 individuals per plant (Costamagna and Landis 2006, Costamagna et al. 2007a, 2008; Walter and DiFonzo 2007), imposing serious limitations to the number of treatments and replicates that can be quan-

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tified efficiently. This leads to trade-offs where the number of experimental units may be limited by the time available for sampling, rather than the desired level of precision.

One way to spend less time sampling without sacrificing the number of experimental units is to develop a reduced sample unit scheme (Southwood 1978, Naranjo and Flint 1994, Moon and Wilson 2009). Reduced sample units, based on within-plant distribution, have been developed for several aphid species (Hummel et al. 2004, Robson et al. 2006, Whitaker et al. 2006). Recently, McCornack et al. (2008) provided an extensive evaluation of 23 potential reduced sample units to estimate soybean aphid whole-plant densities, which was based on direct counts of aphids on different subsets of plant nodes. Based on their analyses, the authors suggested four methods that had the best precision and accuracy to be further developed into sampling plans for soybean aphid. Their data sets included a wide range of plant conditions (i.e., planting dates, host phenologies, plant architectures), demonstrating the generality of the method proposed to follow soybean aphid field populations, mainly toward implementing integrated pest management (IPM) strategies. However, their data sets were based on open field populations, subject to strong top-down control by natural enemies and rain events, which resulted in maximum densities of 1,246 aphids per plant (McCornack et al. 2008) and therefore cannot be directly extrapolated to estimate the higher densities observed under caged conditions. Moreover, exposure to natural enemies significantly affects aphid within-plant distribution (Costamagna 2006), suggesting that the subset of nodes selected for the reduced sample units developed under open field conditions may not be appropriate for caged treatments. Here, using data from four predator exclusion-cage experiments and following the methods outlined in McCornack et al. (2008), we developed and validated reduced sample unit methods to estimate caged populations of soybean aphid. We used a cross-validation technique to select the most accurate method to predict whole-plant aphid density. Finally, we demonstrate that the precision of the reduced sample unit developed here is suitable to detect statistical differences between experimental treatments in two studies testing the impact of predation, and predation in combination with host plant resistance, on soybean aphid population growth.

Materials and Methods

Data Sets. We used four data sets that represent the usual range of aphid densities involved in cage studies, from small initial densities, to very large final densities at the end of the experiments. These were the only data sets available that included detailed node counts, and consisted of weekly counts of soybean aphids at 1) the Entomological Farm of Michigan State University (here after Ento-MSU), Lansing, MI, between 3 July and 8 August 2003 ($n = 80$); 2) the Biodiversity Study of the Kellogg Biological Station-Long Term Ecological Research site (KBS-LTER), Hickory Corners, MI, between 15 July and 5 August 2005 ($n = 47$); 3) the University of Minnesota Research, Outreach and Education (UMORE) Park, Rosemount, MN, between 3 and 26 July 2006 ($n = 37$); and 4) the Sand Plain Research Farm (SPRF) near Becker, MN, between 6 and 20 July 2006 ($n = 48$). Predator exclusion cages were covered by a white, fine no-see-um netting (Kaplan Simon Co., Braintree, MA), buried 25 cm in the soil and tied at the top, supported by an internal cylindrical wire frame (tomato cages) that were 0.4 by 1.0 m (diameter by height). The primarily purpose of this cage is to prevent natural enemies from interfering with aphid growth, and similarly designed exclusion cages have been used previously in this study system (e.g., Fox et al. 2004, Costamagna et al. 2008, Gardiner et al. 2009). At the start of each study, soybean plants were enclosed with predator exclusion cages and contained naturally occurring soybean aphids (Ento-MSU: 10.4 ± 7.0 [mean ± SD] aphids per plant; range, 4–27 and SPRF: 76.1 ± 24.1 aphids per plant; range, 37–114), or were artificially infested with colony-reared aphids (KBS-LTER: 15 aphids per plant and UMORE: 10 aphids per plant). Weekly visual, nondestructive aphid counts were conducted for each node separately, as well as a separate count for the total number of aphids present on all lateral branches (McCornack et al. 2008), for 3 (SPRF), 4 (KBS-LTER and UMORE), and 6 (Ento-MSU) wk after manipulation.

Reduced Sample Unit Validation and Parameter Estimation. To estimate whole-plant density based on reduced sample units, we used the methodology of McCornack et al. (2008) for soybean aphid field populations, focusing on a subset of four methods the authors suggested to use for developing sampling plans. Thus, we assigned numbers for node positions beginning at the upper node, and increasing at lower nodes (see fig. 1 in McCornack et al. 2008). We first showed that the parameters calculated for soybean aphid field populations for the four methods validated by McCornack et al. (2008) were not appropriate for caged populations using the cross-validation method described below. We then estimated unadjusted whole-plant aphid densities for the methods validated by McCornack et al. (2008) using the following equations:

$$E_1\,[N_{MAX'}] = A_{MAX} \times n' \quad [1]$$

$$E_2\,[N_1\text{-}N_{MR}] = (A_1) + (A_{MR} \times (n - 1)) \quad [2]$$

$$E_3\,[N_1\text{-}N_3\text{-}N_{MR}] = (A_1) + (A_3 \times 2) + (A_{MR} \times (n - 3)) \quad [3]$$

$$E_4\,[N_1\text{-}N_3\text{-}N_5\text{-}N_{MR}] = (A_1) + (A_3 \times 2) + (A_5 \times 2) + (A_{MR} \times (n - 5)) \quad [4]$$

where $E_{1\text{-}4}$ are the unadjusted estimated aphid densities for one-, two-, three-, and four-node sample units (see nodes selected between brackets), respectively; A_{MAX} is the number of aphids on the node with the maximum number of aphids within that plant, and n′

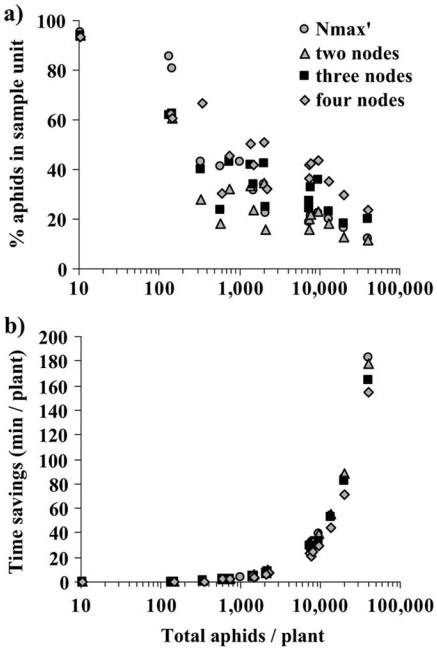

Fig. 1. Sampling savings estimated using reduced sample units. Percentage of aphids in the reduced sample unit versus total aphids per plant, showing a decrease in the percentage of aphids in the sample units as total aphid densities increase (a) and time savings per plant, showing increased savings with aphid density (b). Each point represents the mean of each sampling date for each study (n range, 6–17) for each of the four sample unit methods (N_{Max}; two nodes, N_1-N_{MR}, three nodes, N_1-N_3-N_{MR}; and four nodes, N_1-N_3-N_5-N_{MR}).

is the number of infested nodes on that plant (i.e., nodes with missing true leaves or true leaves without aphids were not counted); A_1, A_3, and A_5 are soybean aphid densities at that specific node position (i.e., node 1 = [N_1], node 3 = [N_3], and node 5 = [N_5], respectively); A_{MR} is soybean aphid density at the middle node in the remainder of the plant. If there were an even number of nodes remaining, A_{MR} was the node closer to the upper node.

To estimate whole-plant densities from reduced sample units, we assessed a linear relationship between them. However, to obtain independent measures of observed versus predicted aphid densities for statistical testing, we needed to remove the common terms in both measures. Therefore, we subtracted the number of aphids used in the reduced sampling unit from both predicted and observed aphid densities, and thus obtained two independent measures of aphid density on the remainder of the plant (i.e., portions not sampled as part of the reduced sample unit). Specifically, following McCornack et al. (2008), we estimated the number of aphids remaining on the plants (E_R) using the following equation:

$$E_R = E_T - \sum A_i \qquad [5]$$

where E_T is the estimated total aphids per plant using equations 1–4, and $\sum A_i$ is the total number of aphids used in the reduced sample unit [i.e., for N_1-N_3-N_5-N_{MR}, $\sum A_i = (A_1) + (A_3) + (A_5) + (A_{MR})$]. Then, we compared E_R with the actual number of aphids observed in the nonsampled nodes (i.e., those excluded from $\sum A_i$), using simple linear regression (SLR), and assessed the performance of the different estimation methods (i.e., equations 1–4) based on the significance of the regression models, their r^2, and the accuracy of model predictions (slope values close to 1). All analyses were performed on natural log-transformed values (with previous addition of 1, to include zeros in the analyses) to meet the assumptions of SLR, using PROC REG (SAS Institute 2001).

To assess the robustness of the parameters estimated by the different sample units (i.e., slope and intercept of SLR), we performed cross-validation analyses. The cross-validation procedure consisted of recalculating the regression parameters with three of the four data sets and using these parameters to predict the excluded data set. This procedure was followed for each data set and for each of the estimation equations. Therefore, instead of arbitrarily selecting a single validation data set, this procedure allows to use all the data sets for validation, resulting in 16 sets of predicted values (i.e., four data sets × 4 sample unit methods), where the observed value was not used in determining the parameters of the predicted aphid densities. This method is particularly appropriate when limited data sets are available, because it maximizes the information obtained from them (Turchin 2003). To calculate the predicted number of aphids per plant (E_C), based on each of the four methods, we used an adjusted equation proposed by McCornack et al. (2008), that incorporates the values of the intercept (a) and slope (b) obtained by SLR, recalculated accordingly for each validation test:

$$E_C = \sum A_i + [e^{(a + b \times (\ln (E_T - \Sigma A_i + 1)))}] - 1 \qquad [6]$$

where all terms have been already defined in equations 1–5. To assess the robustness of each method used to predict each of the data sets, we calculated the coefficient of prediction (Turchin 2003):

$$R^2_{pred} = 1 - \frac{\sum_{t=1}^{n} (Y_t^* - Y_t)^2}{\sum_{t=1}^{n} (\bar{Y} - Y_t)^2} \qquad [7]$$

where Y_t^* is the prediction of the model based on parameters a and b calculated using three data sets, Y_t is the corresponding observation of the experiment excluded from the model, and $\bar{Y}$ is the mean of the observed data. Values for R^2_{pred} closer to 1 indicate very good prediction for the data set excluded from the calculation of the parameters used in the model. Models predicting worse than the observed mean of the predicted data set, have an R^2_{pred} lower than 0 (Turchin 2003).

Comparison of Time Savings. In general, the time required to sample a node without aphids is similar

between nodes (B.P.M., unpublished data). Therefore, time spent sampling a node-based sample unit is heavily dependent on soybean aphid density. To estimate this density-dependent relationship, we counted soybean aphids on digital photographs of soybean trifoliolate leaves. Soybean leaves were collected from caged soybean plants at the SPRF study in 2006, and these cages were separate from those used to develop the reduced sample units in the previous section. A digital camera (model DSC-F717, Sony CyberShot, New York, NY) with a 5× optical zoom was used to photograph aphids on soybean leaves at a fixed distance of 10 cm between the lens and focal point. Even though petioles and main stem were excluded from this estimate of time savings, this conservative approach was used to test the sensitivity of sampling cost for between- and within-plant variability associated with aphid densities on a per node basis. In addition, data obtained by recording the time required to sample open field plants showed a very similar relationship as obtained with this more conservative method (A.C.C., unpublished data). Simple linear regression was used to relate total sample time for counting all aphids on a leaf with associated aphid densities (PROC REG; SAS Institute 2001). The sampling rate (i.e., aphids per s) was used to estimate time savings per sample unit. Specifically, we estimated time savings by calculating the number of aphids not sampled in the reduced sample unit (i.e., total aphids per plant – aphids in reduced sample unit), and dividing them by the sampling rate, obtaining the time saved per plant for each reduced sample unit method.

Case Studies. To test the performance of reduced sampling units, we conducted a full statistical analysis of two experiments comparing observed (i.e., direct counts) versus predicted (i.e., whole-plant densities estimated using reduced sample units) data. Predicted aphid densities were calculated using parameters *a* and *b* from the most robust, reduced sample unit determined by the cross-validation study and equation 6.

The objective of the first study was to test the role of natural enemies in suppressing soybean population growth and consisted of two treatments, a predator exclusion treatment, using the cages described above, and a completely open set of plants, for a total of 18 replicates (Costamagna 2006). We used existing, naturally occurring aphids and paired plants with similar density that randomly received one of the two treatments. Aphids were enclosed on 3 July 2003, and their density was monitored as described above for the next two weeks in a soybean field on Ento-MSU. The experiment was analyzed using analysis of variance (ANOVA) with predator manipulation and blocks of similar initial aphid density as fixed effects, and sampling date as a repeated measures factor using PROC MIXED (SAS Institute 2001).

The second study was designed to test the separate and combined effects of natural enemies and plant resistance to control soybean aphid. We used two pairs of experimental isolines with different levels of antibiosis to soybean aphid: LD05-16519 (susceptible, Susc. 1) versus LD05-16529 (resistant, Res. 1), and SD01-76R (susceptible, Susc. 2) versus LD05-16060 (resistant, Res. 2). Each of the four lines were planted in plots of 9.1 by 3.0 m, which consisted of two internal rows containing the soybean line being tested and two external rows of a susceptible variety S19R5 (NK, Syngenta Crop Protection, Inc., Greensboro, NC). Each treatment was replicated five times per line and arranged in a randomized complete block. As in the previous case study, we established a predator exclusion treatment using the cages described above and an open (uncaged) treatment within each plot. The experiment was conducted on the UMORE Park, Rosemount, MN, where aphids were sampled weekly by node from 3 July to 26 July 2006. We analyzed the experiment with ANOVA and a split-plot design, with host plant resistance (HPR) as the whole-plot factor and predator manipulation as the subplot factor, and modeling sample date as a repeated measures factor (PROC MIXED; SAS Institute 2001). We used Akaike's Information Criterion to select an unrestrictive covariance structure to model error terms (Littell et al. 2006). Significant interactions were explored by slicing by main effects (Quinn and Keough 2002). Because the study was conducted using two pairs of isolines, we tested the HPR effect between the members of each pair using preplanned contrasts. In both case studies, to further test the accuracy of the estimation method, we performed separate paired *t*-test tests between observed and estimated values for individual treatment combinations (PROC TTEST, SAS Institute 2001) and did not adjust for multiple comparisons so as to maximize the detection of potential differences between observed and estimated data sets.

Caged treatments in both case studies were previously used to fit the models for reduced sample units (i.e., Ento-MSU and UMORE data sets). Therefore, following the same logic as for the cross-validation analysis, we used values for parameters *a* and *b* fitted excluding the case study data sets to calculate predicted values (E_C), thus maintaining the independence between predicted and observed data sets. Finally, using the parameters fitted for caged populations resulted in very poor predictions of aphids in the open treatment, as expected (data not shown). Therefore, to estimate E_C for the open treatments in both case studies, we used the parameters fitted for open field aphid populations (see table 4 in McCornack et al. 2008: N_1-N_3-N_{MR}, weighed estimate: $a = 1.17$ and $b = 0.69$).

Results

Reduced Sample Unit Development and Validation. The cross-validation analysis demonstrated that using parameters developed for open field populations (see table 4 in McCornack et al. 2008), resulted in poor predictions for caged populations (R^2_{pred}, N_{Max}: 0.308; N_1-N_{MR}: 0.276; N_1-N_3-N_{MR}: 0.411; and N_1-N_3-N_5-N_{MR}: 0.552). Thus, our results suggest that parameters estimated using open-field populations and within a relatively small range of aphid abundances (i.e., <1,246

Table 1. Results for the SLR between estimated and observed aphid densities on the remainder of the plant using four reduced sample units and data collected from predator exclusion field cages

Sample unit	No. plants (n)	$a \pm$ SEM	$b \pm$ SEM	r^2	F	P
N_{MAX}'	213	−0.73 ± 0.17	0.96 ± 0.02	0.905	2015.8	<0.001
N_1-N_{MR}	213	1.66 ± 0.17	0.80 ± 0.02	0.830	1030.6	<0.001
N_1-N_3-N_{MR}	211	1.43 ± 0.15	0.85 ± 0.02	0.871	1423.0	<0.001
N_1-N_3-N_5-N_{MR}	201	1.52 ± 0.15	0.84 ± 0.02	0.859	1211.6	<0.001

F-tests df = 1, $(n - 1)$.

aphids per plant) do not adequately estimate whole plant aphid abundance of caged populations. Therefore, we proceeded to test the accuracy of reduced sample units to estimate caged aphid populations by fitting new SLR parameters using our four data sets.

Results of the SLRs of the four reduced sample units tested showed highly accurate estimates of nonsampled plant components, with r^2 values >0.83 (Table 1). The most accurate reduced sample unit was $N_{MAX'}$ ($r^2 = 0.91$), followed by N_1-N_3-N_{MR} ($r^2 = 0.87$), and then N_1-N_3-N_5-N_{MR}, and N_1-N_{MR} (Table 1). In addition, aphid density overestimation (estimated by $[1 - b] \times 100$) was very limited, ranging from 4 to 20% on ln-transformed values, and precision around parameter estimates was similar for all four sample units (Table 1). Using the values of a and b showed in Table 1, all four methods provided reasonable estimations of whole-plant density, with R^2_{pred} ranging from 0.81 to 0.92, almost doubling in all cases the R^2_{pred} obtained using parameters from the open field populations estimated in McCornack et al. 2008. However, when individual data sets were tested, the method that provided the most accurate and robust predictions across the four data sets was the three-node sampling unit (i.e., N_1-N_3-N_{MR}), with R^2_{pred} ranging from 0.85 to 0.88 for all the data sets (Table 2). By contrast, $N_{MAX'}$, which was the most accurate method when merging all four data sets together, had very poor predictions for the data sets UMORE and SPRF ($R^2_{pred} = 0.50$ and 0.68, respectively), when those were considered separately by cross-validation. The four-node sampling method (i.e., N_1-N_3-N_5-N_{MR}) showed also very good power to predict individual data sets, although on average was lower than the three-node sample unit, whereas the two-node sample unit also showed lower predictive accuracy (Table 2). Therefore, the cross-validation analysis suggested that the three-node sample unit provides the most accurate and reliable estimate of aphid densities across the four data sets available. In summary, whole-plant aphid density can be estimated using counts of aphids in nodes 1, 3, and the middle node remaining (N_{MR}), and the parameters a and b from Table 1 (1.43 and 0.85, respectively), to solve equation 6.

Comparison of Time Savings. Our data sets covered a wide range of aphid densities, from low densities on the first sampling dates (Ento-MSU: 10.6 ± 7.1; KBS-LTER: 345.8 ± 358.9; UMORE: 147.7 ± 64.4; and SPRF: 601.7 ± 312.6 [mean ± SD] aphids per plant), to peak aphid populations at the final sampling dates (Ento-MSU: 7,568.0 ± 6,306.7; KBS-LTER: 13,367.1 ± 6,866.9; UMORE: 40,975.5 ± 32,360.7; and SPRF: 19,863.1 ± 8,203.7 aphids per plant). Overall, the reduced sample methods involved counting on average 40% of the total aphids per plant ($N_{Max'}$: 41.0 ± 29.2%; N_1-N_{MR}: 32.7 ± 30.0%; N_1-N_3-N_{MR}: 39.4 ± 28.3%; and N_1-N_3-N_5-N_{MR}: 46.9 ± 26.1%; mean ± SD). However, these reductions were much higher on plants with more nodes and higher aphid populations, with <23% of the aphids sampled in reduced sample units using three nodes or less, for plants with >10,000 total aphids (Fig. 1a).

Estimation of sampling costs (i.e., time) were based on 80 leaves (three leaflets per leaf), with densities ranging from 0 to 3,756 soybean aphids per leaf. Not surprisingly, sample time per leaf was positively affected by aphid density ($r^2 = 0.997$; df = 1, 79; $P < 0.0001$), with an estimated intercept of 5.08 ± 5.4 (H_0: $\beta = 0$; $t_{1, 79} = 0.93$; $P = 0.35$), and a sample rate of 3.3 ± 0.02 soybean aphids per second (H_0: $\alpha = 0$; $t_{1,79} = 156.37$; $P < 0.0001$). Using this sample rate we estimated the sampling savings for the four methods, which increased exponentially with aphid density (Fig. 1b). For example, for the three-node sample unit there were savings of 5–8 min per plant at densities between 1,400 and 2,200 aphids per plant, but savings of >29 min per plant when densities were >7,000 aphids per plant (Fig. 1b). Although very large time savings were estimated at larger aphid densities, in

Table 2. Coefficients of prediction R^2_{pred} (Turchin 1993) of cross-validation tests for each data set and the different reduced sample units developed for caged studies (see equation 7 in text)

Sample unit	Ento-MSU	KBS-LTER	UMORE	SPRF
$N_{MAX'}$	0.905 (0.871)	0.781 (0.903)	0.501 (0.923)	0.680 (0.903)
N_1-N_{MR}	0.894 (0.809)	0.731 (0.843)	0.844 (0.826)	0.798 (0.809)
N_1-N_3-N_{MR}	0.954 (0.845)	0.871 (0.875)	0.947 (0.878)	0.940 (0.863)
N_1-N_3-N_5-N_{MR}	0.908 (0.809)	0.919 (0.866)	0.895 (0.875)	0.859 (0.835)

Cross-validation tests used a and b parameters calculated by SLR models excluding the tested data set, and estimates of aphid density using equation 6 (see text). Within parentheses are the r^2 values for all SLR models used to estimate the cross-validation parameters; all SLR models were significant ($P < 0.0001$).

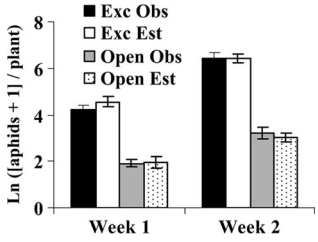

Fig. 2. Effect of predation (case study 1) on the number of soybean aphids (mean ± SEM) tested using field cages, and contrasting observed (Obs.) versus estimated (Est.) counts. Statistical results are presented in Tables 3 and 4. Additional less conservative independent paired *t*-tests between observed and estimated means were not significant (in all cases $P > 0.12$). Exc, predator exclusion cages; Open, uncaged plants.

reality at that level of infestation most leaves are evenly covered and often times is easier to count a subsample of representative leaflets and calculate the total per plant or count groups of aphids (Brosius et al. 2007). These approaches involve less time than the linear extrapolation that we used here to estimate the time required. However, under intermediate levels of infestation, these methods are usually not possible due to the nonuniform distribution of aphids within the plant and therefore the reduced sample unit method proposed here can provide substantial time savings. Finally, we observed similar patterns of time savings for the four sample unit methods developed (Fig. 1b).

Case Studies. *Predation Study.* The treatment means (i.e., predator manipulation and week of observation combinations) calculated for the observed and estimated data sets showed overlapping standard errors and did not differ significantly (all paired *t*-tests, $P > 0.12$; Fig. 2). Accordingly, there was no difference in the ANOVA and slicing tests results using the observed data versus the data estimated using equation 6 with the parameters for the three-node method (Tables 3 and 4).

Predation and Plant Resistance Study. As in the previous case study, most of the 32 estimated treatment combination means (i.e., HPR × predator manipulation × date combinations) showed overlapping standard errors and did not differ significantly for paired *t*-test comparisons from observed means (Fig. 3, $P > 0.05$). Only in three cases (i.e., open treatments in Res. 1 and Res. 2, week 1; and exclusion treatment in Susc. 1, week 2) (Fig. 3), estimated means differed significantly ($P < 0.05$) from the observed means in separate paired *t*-tests. However, these differences were small (Fig. 3) and did not affect the outcome of the overall ANOVA for the experiment, which was almost identical for the estimated and observed data sets (see below; Tables 5–7). The only exception was during the first week of experiment, the observed data suggest only marginally significant differences between predator manipulations treatments ($P = 0.0969$), whereas the estimated data detected significant differences ($P = 0.0097$). This difference may be explained by the smaller dispersion obtained around estimated data. However, because both analyses demonstrate the same pattern in the following weeks, we considered that overall the results yielded the same conclusions. In summary, the estimated data sets yielded the same statistical results as the analysis of the observed data sets for both case studies.

Table 3. ANOVA results from the predation case study comparing the results between observed (Obs.) versus estimated (Est.) counts of aphids: main effects and interaction models

Source	Test	df	*F*	*P*
Predation (P)	Obs.	1, 13.3	120.37	<0.0001
	Est.[a]	1, 15.2	104.71	<0.0001
Block	Obs.	17, 12.9	1.10	0.4395
	Est.	17, 15.0	0.91	0.5764
Date	Obs.	1, 28.7	67.61	<0.0001
	Est.	1, 29.8	84.36	<0.0001
Date × P	Obs.	1, 28.8	5.05	0.0324
	Est.	1, 29.9	7.70	0.0094

[a] Estimated counts were calculated using the three-node reduced sample unit and equation 6.

Table 4. ANOVA results from the predation case study comparing the results between observed (Obs.) versus estimated (Est.) counts of aphids: slicing tests for significant interactions

Effect	Slicing	Test	df	*F*	*P*
Date	Exclusion	Obs.	1, 28.4	54.46	<0.0001
		Est.	1, 29.7	71.3	<0.0001
	Open	Obs.	1, 29.2	18.03	<0.0002
		Est.	1, 30.1	20.65	<0.0001
Predation	Wk 1	Obs.	1, 35	46.61	<0.0001
		Est.	1, 24.8	57.96	<0.0001
	Wk 2	Obs.	1, 35.8	92.88	<0.0001
		Est.	1, 25.6	104.42	<0.0001

Discussion

Our study demonstrates that reduced sample units, particularly a three-node weighed estimation, provided accurate and robust estimation of whole-plant aphid densities under caged conditions. This method resulted in a reduction of 60–77% of the number of aphids counted, resulting in time savings of up to 29 min per plant. We further demonstrated using two case studies that population densities estimated by reduced sample units have enough accuracy and precision to be used in ANOVA models commonly used to analyze data from caged experiments, obtaining nearly identical statistical results as using whole plant counts.

The parameters estimated by McCornack et al. (2008) for open field aphid populations were not appropriate for caged aphid populations. Both the intercept and slopes of the regression of the nonsampled components (i.e., aphids on the remainder of the plant) were always higher for caged populations than for open field populations, reflecting that on average each reduced sampling unit component represents higher aphid numbers on nonsampled components in caged situations. This difference in parameter values obtained between caged and open field aphid popu-

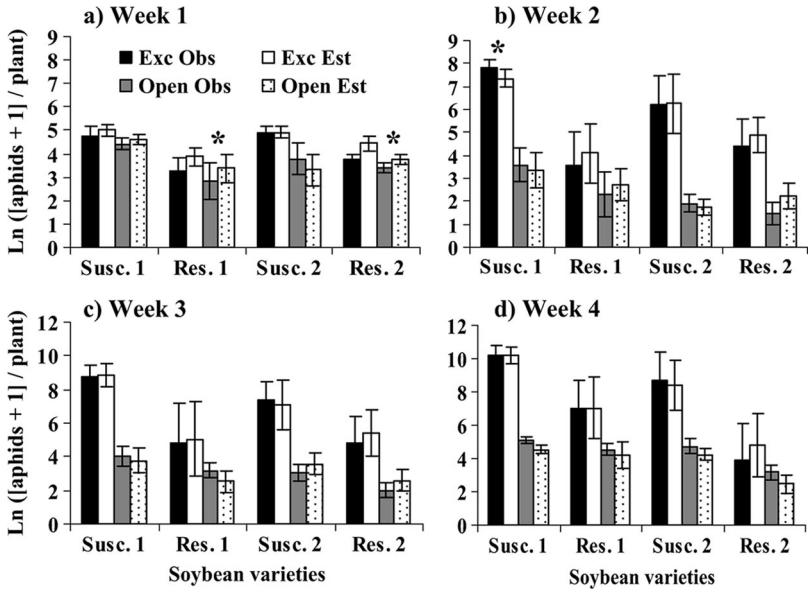

Fig. 3. Effect of predation and plant resistance (case study 2) on the number of soybean aphids (mean ± SEM) tested using field cages and contrasting observed (Obs.) versus estimated (Est.) counts. Statistical results are presented in Tables 5–7. Asterisk (*) indicates significant differences between observed and estimated means by less conservative independent paired *t*-tests ($P < 0.05$). Exc, predator exclusion cages; Open, uncaged plants.

lations can be a product of a different within-plant aphid distribution observed in the absence of predators. Costamagna (2006) found that aphids are proportionally more abundant in the upper (younger) nodes of the plant when predators were excluded but have proportionally lower numbers on upper nodes when exposed to open field predation. Thus, the nodes selected for the reduced sampling unit may represent different portions of the total plant density in open and caged conditions, resulting in different regression parameters. Alternatively, the SLR model used to estimate aphid densities may result in different parameters due to the exponential increase that aphids show in caged conditions (Costamagna et al. 2007b). Finally, it is important to note that our results showed that the parameters calculated for caged populations cannot be generalized to open field populations, which should be estimated using the parameters reported in McCornack et al. (2008).

It is also interesting to note that caged populations were more accurately estimated than open field populations using any of the four methods tested, with overestimation ranging from five ($N_{MAX'}$) to 20% ($N_1 - N_{MR}$) in caged populations (Table 1) versus 22 ($N_{MAX'}$) to 33% (N_1-N_{MR}) in open field populations (see table 4 in McCornack et al. 2008). The higher variability observed in open field estimates and the consequential overestimations also may be the result of higher variability on node to node aphid abundance caused by predation (Costamagna 2006). Although for

Table 5. ANOVA results from the predation and plant resistance case study, comparing the results between observed (Obs.) versus estimated (Est.) counts of aphids: main effects and interaction models

Source	Test	df	*F*	*P*
HPR	Obs.	3, 35.3	3.84	0.0177
	Est.	3, 34.7	3.06	0.041
Predation (P)	Obs.	1, 27.1	24.91	<0.0001
	Est.	1, 28.4	31.53	<0.0001
HPR × P	Obs.	3, 27.1	1.39	0.2677
	Est.	3, 28.4	1.21	0.3254
Date	Obs.	3, 27.9	29.15	<0.0001
	Est.	3, 28.4	14.31	<0.0001
Date × HPR	Obs.	9, 29.1	1.51	0.1912
	Est.	9, 28.5	2.14	0.0590
Date × P	Obs.	3, 27.9	5.92	0.0029
	Est.	3, 28.4	7.10	0.0011
Date × HPR × P	Obs.	9, 28.6	1.16	0.3570
	Est.	9, 28.4	1.03	0.4417

[a] Estimated counts were calculated using the three-node reduced sample unit and equation 6.

Table 6. ANOVA results from the predation and plant resistance case study, comparing the results between observed (Obs.) versus estimated (Est.) counts of aphids: slicing tests for significant interactions

Effect	Slicing	Test	df	*F*	*P*
Date	Exclusion	Obs.	3, 29.9	19.14	<0.0001
		Est.	3, 29.6	12.24	<0.0001
	Open	Obs.	3, 28.3	16.23	<0.0001
		Est.	3, 28.2	9.44	0.0002
Predation	Wk 1	Obs.	1, 17.4	3.08	0.0969
		Est.	1, 17.4	8.42	0.0097
	Wk 2	Obs.	1, 27	21.82	<0.0001
		Est.	1, 27.6	28.22	<0.0001
	Wk 3	Obs.	1, 28.9	20.22	0.0001
		Est.	1, 29.6	18.15	0.0001
	Wk 4	Obs.	1, 28.6	21.11	<0.0001
		Est.	1, 29.1	25.87	0.0001

Table 7. ANOVA results from the predation and plant resistance case study, comparing the results between observed (Obs.) versus estimated (Est.) counts of aphids: contrasts between isoline pairs

Contrast	Test	df	*F*	*P*
Res. 1 vs. Susc. 1	Obs	1, 34.9	6.77	0.0135
	Est	1, 34.5	6.36	0.0165
Res. 2 vs. Susc. 2	Obs	1, 35.6	3.86	0.0573
	Est	1, 34.8	1.96	0.1709

experimental studies an overestimation of 15% in aphid density (i.e., for the N_1-N_3-N_{MR} method) may seem too high to contrast treatments, our two case studies demonstrated that this level of precision produced identical statistical results and biological conclusions as the observed total counts per plant.

To our knowledge, this is the first study that demonstrates that reduced sample units developed for field populations of insects cannot be directly used in field cage experiments. Reduced sample units at the within-plant level have been developed for several pest species in uncaged conditions (von Arx et al. 1984, Naranjo and Flint 1994, Gould and Naranjo 1999, Hummel et al. 2004, Arnó et al. 2006, Robson et al. 2006, Whitaker et al. 2006) and recently to field populations of soybean aphid (McCornack et al. 2008). However, predator exclusion cages have been extensively used to quantify the impact of natural enemies on different pests (reviewed in Kidd and Jervis 2005), including several aphid species (Chambers et al. 1983, Hopper et al. 1995, Mohamed et al. 2000, Colfer and Rosenheim 2001, Brown 2004). Furthermore, numerous recent studies on soybean aphid tested the impacts of natural enemies using field cages (Fox et al. 2004; Costamagna and Landis 2006; Desneux et al. 2006; Brosius et al. 2007; Costamagna et al. 2007a, 2008; Donaldson et al. 2007; Gardiner and Landis 2007; Rhainds et al. 2007; Schmidt et al. 2007; Chacón et al. 2008; Gardiner et al. 2009). Field cage studies are also useful to determine aphid population growth parameters (Costamagna et al. 2007b, Matis et al. 2009); test the role and interactions among different natural enemy guilds (Colfer and Rosenheim 2001, Cardinale et al. 2003, Snyder and Ives 2003, Chacón et al. 2008, Costamagna et al. 2008); and test the effect of bottom-up factors, such as host plant resistance (Messina and Sorenson 2001, Hill et al. 2004b, Mensah et al. 2005) and nutrients (Walter and DiFonzo 2007) in pest control. The rapid development of host plant resistance against soybean aphid (Hill et al. 2004a,b; Li et al. 2004; Mensah et al. 2005; Hill et al. 2006a,b; Hesler and Dashiell 2007; Hesler et al. 2007) and their potential tritrophic interaction with natural enemies (Lundgren et al. 2009) also warrant further studies involving field cages in this system that can benefit from applying the reduced sample unit developed here.

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