PERSPECTIVES ON SAMPLING FOR *LYGUS* IN COTTON: APPLICATIONS OF QUADRAT-BASED SAMPLING SCHEMES

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Abstract

Objective assessments of row crop attributes are necessary for optimal crop management. This study primarily demonstrates the use of line-intercept sampling (LIS) to estimate two row crop attributes; namely, stand counts and populations of *Lygus* spp. in cotton. For either type of count, prior to canopy closure, transect lines of known length are randomly positioned at right angles to imaginary reference baselines that lie parallel to the rows. Ideally, the transect line and baseline lengths are selected to define a reference area that comprises one land acre, or for enhanced brevity of sample effort, quarter-fractions thereof. Sample information is collected from constant size quadrats intercepted by the transect lines. A quadrat is a small, rectangular area centered on a row that is bisected (i.e., encountered) by the transect line and corresponds to either the length of a yardstick or drop cloth. Attention is directed toward estimating crop attributes measured from a series of adjacent quadrats having fixed row distances; therefore, several transect statistics may be estimated using simple formulae. One particular statistic of interest discussed here is the attribute totals (i.e, the number of plants or plant bugs) per acre. Alternately, a visual technique for sampling plant bugs after canopy closure is also described. This method uses non-adjacent quadrats where the size of each is now two adjacent rows, each 9 *ft* long. Sub-samples of 5 randomly drawn plants per quadrat are visually examined, using terminal scouting or whole plant visual samples. Each terminal or plant is counted as infested if at least one plant bug life stage is found. A simple 'pen and paper'computer is described that provides the estimate of percent infested plants with this '2 x 9' or 'stop' sampling method. For both sampling methods, for either plant bugs or stand, the user should stratify the samples where crop phenology are similar. Estimates of each strata should not be combined, in most instances, into a final average.

Introduction

This article describes how to combine the drop cloth with a line-transect method to produce a superior sampling technique for nymph and adult stages of *Lygus* spp. in cotton prior to canopy closure. Once the canopy has closed, the scout should use the visually based, non-adjacent quadrat method described in this article.

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Each quadrat type will be briefly described and examples that illustrate the use of each quadrat type are provided. The first example shows how the transect method can be used to estimate stand, or the density of cotton plants per acre. The second example features the marriage of line-intercept sampling concepts with a traditional plant bug sampling tool, the drop cloth, to estimate plant bugs per acre. The final example shows how two kinds of quadrats, those in series and those not in series, can be combined to create a visual, whole-, or partial-plant (i.e, terminal) sampling method that estimates 'minimum' numbers of plant bugs per acre. This method must be applied once the plant canopy laps and makes difficult the use of a drop cloth. Assessing the magnitude of plant bug damage with either scouting method is also briefly described.

The use of a sweep net, another traditional scouting tool, is not prominently featured because, in my opinion, the two quadrat-based plans are more reliable; particularly, at sparse densities.

This discussion mentions only key points and focuses upon application of these scouting techniques for decision-making with production fields. In contrast, these methods have not been used in small plot settings. The methods should still apply, but the concern is that small plots can be over-sampled and introduce a bias; especially, if the time interval between samplings is short. Further details or narrative, especially those of a statistical nature, can be found by consulting the listed references, or contacting the author.

Methods and Materials

Background Information

To best use these (or any other) sampling technique one should have clearly in mind the boundaries of the area from which sample data are to be collected and to which management decisions will apply. Frequently, a field can be sub-divided into smaller areas, especially if the field conditions vary (e.g., stage of plant growth, fertility, or moisture). When the field is divided into similar or homogenous blocks, each block is sampled as if it were a unique field. In this discussion, each smaller sub-unit of a field will be called a management unit and is a specified area of land on which decisions are made and actions taken (Williams et al., 1995). The key concept that defines a management unit is that actions taken on this unit are carried out uniformly (See Williams et al., 1995, for further discussion). From the perspective of sampling, it is important that sample data be collected and kept distinct from sample data where soil type and fertility, water management, emergence date and other traits of crop phenology differ. To fail to do so, makes difficult the identification of pattern on the temporal and spatial changes of pests. Whenever patterns in space and time of pest dynamics can be identified, the task of choosing appropriate pest management strategies and tactics becomes easier. If possible, remote sensing techniques can be used to identify management units. However, if such images are not available, be practical. For example, the stand density along with plant height (or differences in growth rate) as related to the slope and aspect of the terrain, soil type, or drainage can be used to define the sample strata, or management units.

Another important issue is to remember that both the identification of spatial pattern and density are heavily influenced by the size of the sample unit. Detecting whether a population is clumped or randomly dispersed is strongly determined by the sample unit size (Ludwig and Reynolds 1988; Wilson and Room 1983). With the quadrat based plans described here, the sample units more strongly reflect units of area of land rather than a plant or small collections of plants. In fact, both plans assume that the field, or more particularly, the management unit have been gridded into quadrats of the size used by each plan. The random sample is obtained by drawing these sample units, not random samples of plants, and provide the feature of expressing counts as numbers per acre. However, due to a strong tradition of using plants, or sets of plants, as the sample units, both plans can report the results as 'percent infested plants' without loss of applicability or generality. Another property related to the size of the sample unit is the ease of detecting insects. For example, as the sample unit size becomes smaller and approaches the size of a single plant, the chance that a randomly drawn sample unit will be found with an insect of a particular kind or life stage decreases as the abundance of that insect decreases. However, the ease at which a sample unit can be searched becomes easier as size becomes smaller. Conversely, as the sample unit increases in size, the chance that an insect occurs somewhere inside the sample unit increases, but at the penalty of having to spend more time and effort examining plant biomass residing within the sample unit. More details of this phenomenon, along with graphical displays showing the relationship between density, degree of spatial clustering and sample unit size can be found in Willers et al. (1990).

The analysis of spatial pattern with line-intercept sampling designs is particularly efficient. The technique involves a transect line having both width and length; therefore sample data obtained from each row crossed by a transect line can be analyzed by quadrat-variance methods (Consult Ludwig and Reynolds (1988) and Pielou (1977) for details). For now, my opinion, after using these spatial analysis techniques (JLW, unpublished), has steadily grown toward the view that many agricultural insect pests follow a random spatial pattern that is either fine- or coarse-grained (See Pielou 1977). The graininess of the random spatial pattern is a function of the species and the time of year and duration of establishment upon the cotton crop. Crop phenology also influences the grain of the random spatial pattern. Practically speaking, this kind of knowledge is invaluable for understanding the relationship between the frequency of sample units that are classed as uninfested or infested. To examine this issue further, consider another property of insect sample data.

It is a fact that as the mean number of insects per sample unit (of any size) increases, the proportion sample units occupied increases until all units are infested (See Wilson and Room 1983 and Wilson et al., 1989). In practice, this property implies that distance between plants decreases as the number of plants infested with at least one insect increases. For example, if one assumes a fine-grained, uniform spatial pattern, a 38" row spacing, and 40,000 plants per acre the distance between occupied plants at 400 insects per acre (or 1%) is 39.39 *ft*. At 12,000 insects per acre (or 30%) the distance between plants is 1.15 *ft*. The relationship between distance and density asymptotically decreases fairly sharply, and begins to level off at 12, 000 insects per acre. The logic of this relationship is straightforward. Simply assume that the number of linear row feet per acre is one continuous line. Next, divide the total number of linear row feet by different densities of insects per acre. The result will be uniformly sized line segments that represent the 'average' distances between individuals at a density of one per plant. Interestingly, these distances approximate the average (i.e., the expected value in a statistical sense) distances between individuals that follow fine-grained, randomly dispersed spatial pattern. These line segment lengths can be easily graphed against the corresponding densities. (The reader is urged to do this simple exercise for personal instruction and benefit.) It should also be remarked that a biological reality related to this association between distance and density (and unlike the simplifying assumption of only one insect per plant just given above) is that the percent of multiply occupied plants increases as the density of insects increases.

Hence, for scouting purposes the multiple occurrence of insect pests among sample, or even sub-sample (e.g., a plant) , units is partial evidence of a severe infestation. To conclude with certainty that a serious problem exists throughout the management unit, knowledge about the grain of the spatial pattern needs to be available. Otherwise, the proper interpretation of the meaning of multiple occurrences of insects on individual plants may not be reached. For example, if the spatial pattern is fine grained, one can conclude with confidence that the entire management unit is occupied. On the other hand, if it is coarse grained, then only localized regions of the management unit are occupied . Other regions are relatively free of insects when the spatial pattern is coarse grained. The impact upon pest management decisions between the two types should be obvious. Pest management options vary dramatically between partial or complete spraying of the entire management unit. Several examples appear later in this article that illustrate how to infer the granularity of the insect spatial pattern from sample data.

A final comment about the sampling methods described in this article needs mentioned. If the plan makes use of sub-samples of single plants, the response variable of interest is whether or not the plant is occupied with at least one insect of a particular species and life stage. Plants are binomially classed (Wilson and Room 1983) as occupied, that is, as a ‘yes’ or ‘no’ irrespective of how many individuals of the same kind are present. Multiple occurrences of the same insect and life stage are not counted, unless the sampler desires to so. If a record of multiple occurrences is required, the sample data are recorded into classes as ‘1+, 2+, 3+, ..., etc.’ to reflect sample resolutions of more than one, more than two, or more than three, and so on. The data are then analyzed by class. Note, however, that when the LIS sampling method is combined with a drop cloth, the sample data recorded are numbers per drop cloth per sampled row. On the other hand, for LIS sampling using either whole or partial visual plant samples per length of row, the earlier comments about the binomial classification of the sample data also apply.

<u>Line-Intercept Sampling and Drop Cloth Method (or Quadrats in Series)</u>

The line-intercept method (LIS) was adapted from forestry and wildlife biology (Kaiser 1983; Lucas and Seber 1977; McDonald 1980, 1991) where it has a long standing history. It is especially useful to estimate the stand (i.e., number of plants per acre) of cotton fields. (Also, it can be used to make replanting decisions whenever severe row skips occur (Willers et al., 1992).) The method is based on an imaginary reference baseline that runs down the center of a cotton furrow (forming the long side of a rectangle), and a transect line that originates from a point on the baseline and runs across the rows (forming the short side of the rectangle). The larger rectangle thus formed is scaled to one acre in size (43,560 square feet), and forms the basic reference area where an estimate of stand, or another attribute like plant bugs per acre, are made. In all cases, the transect line must be perpendicular to the row direction; it should be straight and not curved or zig-zagged. A feature of particular interest is the fact that these two lines create an inherent, but simple, randomization scheme for collecting sample data without bias.

For insect scouting, I recommend LIS with visual methods for observing plant bugs only in cotton early in the season when plants are small (Williams et al., 1995). If the LIS method is used with a drop cloth the interval of applicability and timeliness are both increased. Discussing the marriage of LIS methods with a drop cloth for plant bug scouting is an emphasis in this article.

A computer program, using a Windows® graphical user interface is being built to perform the computational steps for either stand or insect attributes (Akins et al., in preparation). This program, called *CASA* (for <u>C</u>omputer <u>A</u>ssisted <u>S</u>tand <u>A</u>nalysis) should be available by the 1999 cropping season. However, the *CASA* software is not required for use of the technique. (In fact, spreadsheet programmers can build worksheets that provide LIS estimators (See McDonald 1980, 1991, or Willers et al., 1992 for the necessary equations).)

<u>Concepts and Terminology</u>

In the following discussion, it is important to distinguish between the concepts of "*distances (or lengths) of row*" and "*row spacings or widths*". Many traditional definitions used in LIS have been altered to conform to cotton crop terminology; specifically, I have reversed the definitions of length and width for the two-dimensional objects intercepted by transect lines without changing the applicability of formulas (see Eqn. [1]). Another difference from standard LIS methodology is that two-dimensional ‘objects’ do not actually exist in cotton. These two-dimensional objects (or particles) in traditional LIS applications are items such as shrubs, rocks, holes in blocks of cheese, protozoans on a microscope slide, etc. These objects are sampled, or counted, only when they are intercepted by a transect line running through the study area. In the scouting of cotton for plant bugs or stand density, these objects now represent small rectangles within the field whose dimensions are the furrow width by the drop cloth or yard stick length. Given this fact, the traditional terms of two-dimensional "objects" or "particles" intersected by a transect line are now replaced by the term "quadrat". We emphasize that quadrat's are artificial sampling units (Ludwig and Reynolds 1988) improvised for convenience. However, these artificial units have two unique properties that enhance the precision of the method. First, as the transect line crosses more and more rows, sample units of different sizes are dynamically created. Secondly, as the sample unit size increases, the chance that a sample line will encounter an insect approaches a certainty. Thus, the correspondence between the LIS method and some sampling properties just mentioned above is quite close. Typically, for an 8 row sample line this chance is 100 % for populations of plant bugs as rare as 1.4% or 573 plant bugs per acre and follow a fine-grained dispersion pattern.

A "drill" is a row of planted seeds centered between two furrows. In cotton, quadrats are derived from fixed-distances of row along the drill. The fixed lengths of row have plants present and if any gaps exist between plants, these are smaller than a specified minimum length, typically 3 *ft*. If the gap is greater than 3 *ft* a zero should be recorded. The drop cloth or yard stick length comprises the length dimension, $\boldsymbol{l}$, of the quadrat. Similarly, the furrow spacing comprises the width dimension, $\boldsymbol{w}$, which is a constant in solid stands (i.e., all rows are planted at equal spacings).

Let $\boldsymbol{L}$ be the length of a single, randomly located transect line originating on a randomly chosen baseline of length $\boldsymbol{W}$ along the reference area, $\boldsymbol{A}$. The variable, $\boldsymbol{n}$, is the maximum number of rows used to establish a transect line of length $\boldsymbol{L}$. Note that the product of $\boldsymbol{L} * \boldsymbol{W} = 1$ land acre, or 43,560 ft^2. However, if a shorter length (either $\boldsymbol{k} = 24$ or

16 or 8 rows) is selected for the transect line length, then the study area can be subset into smaller areas; for example, 3/4, ½, or 1/4 *ac* sample tracts. The length of the transect line and baseline must be established in order to estimate the stand or plant bug counts using LIS. From these specified dimensions, it is possible to build *a priori* a table of constants to estimate totals per acre (See Table 1). These constants can be derived from logic, or application of the equations found in McDonald (1991) and presented again in Willers et al. (1992). In cotton, the fundamental length of a transect line corresponds to the planter configuration that planted the crop. The maximum length of a transect line is some multiple of the number of planter passes sampled and meets the constraint (along with the baseline length) that the area of the reference rectangle be 1 land acre. For example, the transect line's maximum length is 101.33 *ft* (32 rows) if an eight-row planter is set to a 38 inch row spacing and four planter passes are sampled (8 rows x 4 passes x 38 *in*/furrow ÷ 12 *in*/*ft*). If it is convenient to let four planter passes define a maximum transect line that spans one acre, then by definition the baseline length is 429.9 *ft* (43,560 ft^2 ÷ 101.33 *ft*). Similar calculations apply for other row spacings that are different than 38 *in*. The process of moving across rows gives this method its strength by capturing the variability in the crop due to planting irregularities or other causes that occur. The accuracy of the estimate depends on the variability of the stand, and the length and number of transect lines used to collect the data.

In row crops, the line segment that represents the intersection of the transect line and the i^{th} quadrat is (by definition) the span between furrows, $\boldsymbol{w}_i$. The length, $\boldsymbol{l}_i$, is the dimension (i.e., the drop cloth or yard stick width) of the i^{th} quadrat perpendicular to the transect line. The subscript $\boldsymbol{i}$ (i = 1, 2, 3, ..., ≤ $\boldsymbol{n}$) counts the number of individual (i.e., sampled or encountered) quadrats intercepted by the transect line.

Also, if more than one transect line is used within a study area (an issue ultimately left up to the user), quadrats from one line should not overlap quadrats associated with another line. In either instance, if overlap occurs, the transect lines cannot provide independent samples (Anderson et al., 1979; McDonald 1991).

When collecting a sample, the observer first locates a baseline's origin and then moves to the point on the baseline where the transect line begins. Next, the transect line is traversed using either the yard stick or drop cloth on each row.

To estimate the attribute totals per acre, $\hat{\boldsymbol{Y}}$, for all $\boldsymbol{y}$ in $\boldsymbol{A}$, a simple equation
can be used. The sample data collected from the quadrats along a line whose length is a chosen number of rows is plugged into the following equation:

$$N \sum_{i=1}^{n} (y_i/l_i) \; = \; W/l \sum_{i=} \qquad \textbf{(1)}$$

If the transect line length is < $\boldsymbol{L}$, then the result $\hat{\boldsymbol{Y}}$ needs to be adjusted by a correction factor which is the ratio, $\boldsymbol{L}/\boldsymbol{k}$. The expression estimates the number of plants or plant bugs per acre.

Two by Nine Visual Scouting Method

The two by nine, or stop, sampling method uses a sampling unit also called a quadrat, but is now defined as two adjacent 9-foot sections of row (hence, the origin of the name). Unlike the smaller quadrats of LIS which occur adjacent to one another, these are not adjacent to each other. Several stops are randomly located in each management unit of the field. Typically, three-to-seven quadrats are sampled per management unit, but in severe infestations only one stop may need to be sampled. Allowing a range in the number of quadrats provides flexibility to handle all situations encountered in the field, but also requires judgement on the part of the scout. With experience, scouts will develop an intuitive feel for the proper sampling intensity based on what they encounter in the field (I will show in a moment how the data can assist in this assessment).

Five representative plants are selected alternately from the two rows of the quadrat. The observer has complete freedom to choose which plants to sample. In fact, the highest quality plants preferred by the pest in the stop are the best ones to select. The method guards against observer bias in the random selection of the stop. Therefore it is not necessary to impose the additional requirement that the sub-sample units also be randomly selected within the stop. These sub-sample units (i.e., terminals or whole plants) are visually examined for plant bug adults or nymphs. Square bracts should be opened since adults and nymphs often hide inside. The time required to sample each of these kind of quadrats for plant bugs is a function of the sampler's skill and whether he/she visually inspects just terminals or whole plants. Quadrats (as is true for LIS samples) should be separated in the management unit by at least 150 feet and located no less than 100 feet from the edge of field.

Results

Example 1- Stand Counts (LIS)

To apply the technique, a starting point for sampling is chosen at random in a management unit. This point represents the intersection of the baseline and the transect line. (Interestingly, with the increased emphasis upon GPS (Global Positioning Systems) these points can be pre-selected and navigated to with the use of a real-time GPS receiver.) Sampling for the attribute of interest is next conducted along the transect line for the specified width and across the required number of consecutive rows until the end of the transect line. For example, one way to estimate the number of cotton plants per acre is to count the number of plants in each 3 *ft* section of row (using a yard stick as a guide) crossed by a 32 row long transect line, recording each value on a data sheet (These sheets are prepared for you in Williams et al., 1995).

For a single transect line, plant counts from three-foot sections of row over 32 rows are taken, providing a series of numbers similar to: 9, 6, 5, 10, ..., 11, 7, 10, and 8. These numbers are summed across rows (e.g., $\Sigma y_i = 288$) and multiplied by the baseline length (**W**) of 429.9 feet (e.g., 288 x 429.9 = 123,811). This value is divided by the length of row examined ($\boldsymbol{l} = 3\ ft$) to yield the number of plants per acre, in this case 41,270. To assess the variability of the estimate, the process is repeated using four or more transect lines in the management unit. These results can be averaged to obtain an estimate of the best representative value for the management unit where the sample lines are placed.

It is not always necessary to use a transect line four planter passes long. If fewer than four planter passes are used in the sample, adjustments in the calculations provide estimates of plants in quarter-acre increments. In the example above, a transect line of eight rows represents a sample of one-quarter acre, 16 rows represent a sample of one-half acre, and 24 rows a three-quarter acre. In these instances, simply apply Eqn. [1] for the data obtained from a shorter line and multiply the result by the ratio of the maximum line length to the actual line length used. For example, the result from an 8 row sample is multiplied by the correction factor of 4, or the ratio 32/8.

Alternately, the estimate can be easily obtained if a table of constants appropriate for the transect line length and width is available (See Table 1 here and in Williams et al., 1995). Sample counts for stand, taken within a particular length of row for different lengths of a transect line are simply summed across all sampled rows. This sum is multiplied by the correct value from the table to directly convert these data to per acre estimates.

The user has the choice to define the line length per sample, the length of row segment examined on each row crossed by the transect line, and the number of lines per management unit (Willers et al., 1992; Williams et al., 1995). Presently, I believe that four, eight row long lines per management unit is more than adequate (in most cases) to estimate the stand of the crop.

Example 2- Plant Bugs (LIS and Drop Cloth)

Estimating the number of plant bug adults and nymphs per acre follows in similar fashion. The resolution of the desired sample determines the length of the transect sample line. For example, most drop cloths are 3 *ft* wide, and with a 38" row spacing without skip rows, an eight row sample can detect up to 573 plant bugs per acre. A 16 row sample with the same conditions can detect up to 286 plant bugs per acre. By comparison, if a 5 *ft* drop cloth could be used, a 32 row sample could detect up to 86 plant bugs per acre. Thus, the choice of drop cloth width and length of successive samples across adjacent rows determines the sensitivity of the method to detect low densities (See Table 1 in Williams et al., 1995). I know of no other method that can operate at such low absolute densities in sampling for plant bugs.

Presently, in scouting for plant bugs I find an 8 row sample quite adequate, perhaps 16 rows at most. Frequently, 3 lines of this length per management unit will be sufficient provided the field is stratified into management units. The sample time per line, using an assistant to record the called out counts from the surface of the drop cloth, is up to 4 - 5 minutes per 8 row sample. This includes recording the number of plants per row, the number of plant bug adults and nymphs (by instar), number of beneficials by species and number of abscised fruiting forms shaken off the plants. If all this other information is not necessary, the amount of time per sample line can be markedly decreased.

As the plant bug densities increase and are obviously above a treatment threshold, the line length can be shortened and the estimate similarly scaled by the fraction of rows (***f***) sampled with reference to an ideal 8- or 16-row sample (***k***). For example if 5 plant bugs are found from a 2 row drop cloth sample, the estimate (using the scaling factor for an 8 row sample as found in Table 1 and a secondary correction factor $\boldsymbol{k}/\boldsymbol{f} = 8/2$) is 5 * 573.2 * 8/2 = 11464 bugs per acre. The same problem solved with Eqn. [1] and use of the primary correction factor ($\boldsymbol{L}/\boldsymbol{k}$) results in $\hat{\boldsymbol{Y}} = 429.9/3 * 5 * 32/2 = 11464$ bugs per acre. With experience and judgment, the sampler will learn how to balance the length of the sample line against the value of the information needed to make a management decision.

Several additional examples are now mentioned. First, if an 8 row sample is zero for all 3 *ft* row lengths sampled along the line, the estimate (using the constants of Table 1 or Eqn. [1]) of plant bug density is zero, but is better thought of an interval bounded by the limits $0 \leq \hat{\boldsymbol{Y}} < 573.3$ per acre. For an answer of exactly zero, no line of any length can be provided to actually conclude that the field is completely free of plant bugs.

If an 8 row sample, provides the sample data of $\boldsymbol{y}_i$ = 0,0,0,1,0,1,0,0, then by Eqn. [1] and use of the correction factor ($\boldsymbol{L}/\boldsymbol{k}$), the estimate is $\hat{\boldsymbol{Y}} = (429.9/3)*2*32/8 = 1146.4$ bugs per acre. Alternately, using the scaling constants found in Table 1, the estimate is $\hat{\boldsymbol{Y}} = 2 * 573.2 = 1146.4$ bugs per acre. The occurrence of numerous zeros for these two example lines suggest a coarse-grained spatial pattern of plant bugs throughout the management unit.

The last example suggests both a fine-grained spatial pattern and a severe plant bug problem. Here, drop cloth samples of an 8 row line provide the following data, $\boldsymbol{y}_i$ = 2, 2, 2, 0, 2, 0, 2, 3. Using the scaling factor from Table 1, $\hat{\boldsymbol{Y}} = 573.2 * 13 = 7451.6$ bugs per acre. It is left to the reader as an exercise to obtain the same result using Eqn. [1] and the correct correction factor.

Example 3-Two by Nine Sampling Method (Visual counts of Plant Bugs)

The following example illustrates the calculations required to estimate the percentage of cotton plants infested with plant bug adults and nymphs. Plants with more than one plant bug adult or nymph are counted only once; for example, if five plants are examined, and one of the five has two plant bugs, then only a '1' is tallied. Practically, this means that in estimating the number of bugs per acre, only the lower limit of the total possible number present is estimated. I adopt this convention because of the time savings that it provides. Otherwise, multiple occurrences of plant bugs on the same plant are only recorded if desired by the observer.

In this example, five plants in each of three quadrats (also called 'stops') are sampled. Visual counts of infested plants per number of plants inspected per stop are 1-in-5, 2-in-5, and 1-in-5. To estimate the infestation rate of plant bugs, the number of plants with bugs is divided by the total number of plants sampled (e.g., 4 ÷ 15). This calculation suggests that 26% of the plants are infested with plant bugs. Although this is a standard estimate applied in scouting, this estimate can be further assessed with this sampling design. On face value, this estimate neglects the fact that three of three stops in the field had plant bugs -- valuable information that can be used to assess the persistence of the infestation in the field.

An easy method to infer the persistence (granularity) of plant bugs in this field, is to take the square root of the following ratio: the number of quadrats with plant bugs divided by the total number of quadrats sampled. In our example the value is 1.0 ($\sqrt{3 \div 3}$). This proportion is multiplied by the proportion of infested plants (0.26) to yield an adjusted infestation rate of 26%. The adjusted estimate is used in decision-making. Here, given that the first estimate is unchanged by the use of the second value, we can make a decision with increased confidence that plant bugs are commonly abundant in this field.

To illustrate this point further, let's consider an example from one other extreme. Suppose that the data had been, 4-in-5, 0-in-5, and 0-in-5 plants. This gives the same infestation level (26%) as before (4÷15), but now the second quantity is 0.57 ($\sqrt{1 \div 3}$), instead of 1.0. Only one of three stops examined were found to have plant bugs. When 26% is multiplied by 0.57, an estimate of 14.8% is obtained. This estimate also suggests that control measures should be implemented, but a closer look at these data suggest that more thought should be given to this situation before spraying the management unit.

First, it is observed that all bugs were discovered from one stop and in a large percentage (4-in-5 plants). If this one stop is a reasonable estimate of the actual numbers to be expected in the field, common sense, experience and simulation studies (Willers et al., 1990a,b) indicate that the chance of having two stops with zeros is quite small, if not impossible. What is really happening here? Several questions need to be asked. Is the individual reporting the data highly trained, or a new scout? If he is new, perhaps thought should be given to further training, and have an experienced scout verify the status of this field. If the person is experienced and highly qualified, did the data from the one stop come from under the center pivot with the two zero-valued stops coming from areas not covered by the pivot? If the answer to this question is yes, the field was not properly stratified into management units before the samples were collected. The answers to these or other questions should lead to better management decisions. Certainly, one may want to collect more samples before deciding to treat.

The example discussed thus far has illustrated how to visually inspect individually stops and selected plants within each quadrat (rather than individual plants or all plants from non-adjacent lengths of row) to estimate pest abundance, information on spatial pattern, and deduce if the data obtained is reliable or not.

I now bring the two sampling plans together to estimate the number of plants with at least one plant bug adult or nymph per acre. One does not necessarily have to do the following to make a decision, but the result is useful to help interpret data and form in one's mind better economic thresholds in mid- to late-season cotton. The row spacing is 38 inches and there are no skip rows.

First, from LIS samples for stand, estimate the average number of plants per stop. Each stop has 18 *ft* of row. From LIS data given earlier above, it was found that 288 plants were counted from 32, 3 *ft* lengths of row; therefore, 288 plants per 96 *ft* of row is equivalent to 3 plants per *ft*. Thus on average, a stop is expected to contain 18 * 3 = 54 plants. Next, calculate the number of stops per acre. Each stop has 18 *ft* of row; therefore, 13,756.8 linear *ft* per acre divided by 18 linear *ft* per stop gives 764.6 stops per acre. Then, obtain the total number of plants found with plant bugs from all plants inspected from the 3 stops (e.g., 4-in-15) and the number of stops found infested of the total number stops inspected (e.g., 3-of-3). Now, solve the following expression:

$$\left(\frac{4\ \textit{infested plants}}{15\ \textit{plants}}\right)\left(\frac{54\ \textit{plants}}{\textit{stop}}\right)\left(\frac{764.3\ \textit{stops}}{\textit{acre}}\right)\left(\sqrt{3/3}\right)$$

When solved, the result of 11,005.92 infested plants per acre is obtained which is an estimate of the lower limit of the total number of bugs per acre. The value is a lower limit because of the requirement that a plant was classed as infested if *at least* one bug was found. Finally, if desired, divide the number of infested plants per acre by the number of linear feet per acre (13,756.8), then multiply by 100 to provide the result that there are at least 80.003 plant bug infested plants per 100 *ft* of row.

Determine Percent Square Set

Count the total number of squares in the top 5 nodes of each of the 5 plants from each stop across the management unit (using no more than 2 squares per fruiting branch). Assuming that fruiting begins on the 6th node the following pattern results:

6th node cotton should have 5 squares/5 plants,
7th node cotton should have 10 squares/5 plants,
8th node cotton should have 20 squares/5 plants,
9th node cotton should have 30 squares/5 plants,
10th node cotton should have 40 squares/5 plants,
After the 10th node, maintain 40 squaring sites/5 plants.

Therefore, the (actual number squares / potential sites) * 100 = % Square set. I use this traditional method (Williams et al., 1991, 1995) to assess plant bug damage, with the exception that I record and estimate the square set for each stop, average all the stops, and then subjectively look at all the data to determine the consistency of the average estimate of square set.

With the LIS and drop cloth sampling method, the number of abscised fruiting forms shaken off the plant and classed as plant bug damaged can be used to estimate the number of damaged forms per acre.

Discussion

Two important points have to be kept in mind to appreciate the use of quadrat sampling schemes. First, consider the ratio (percentage) between the size of the sample unit and the most convenient area of field to which the sample is expected to apply. Some useful ratios to consider are: 1 plant/40,000 plants/acre, or 0.0025%; 3 *ft* of row/13,756.8 row-ft/acre, or 0.022%; 1 quadrat/764.3 quadrats/acre, or 0.131%; and finally, 1 plant/54 plants/quadrat, or 1.85%. Notice that the percentages are increasing as one moves from left to right. Given that insects are difficult to search for at sparse or low densities, one would like to have a sampling plan that allows as large a fraction of the population to be sampled as possible. This is achieved with the 2 x 9 sampling method where two sizes of sample units are actually used at the same time; specifically, several single plant sub-sample units nested inside a second, but larger sized sample unit called a quadrat.

In a somewhat different sense, the series of quadrats formed by LIS offer another advantage. Here, the sample unit size is dynamically increasing as a function of transect line length. Different sized sample units can be created by collecting together sets of adjacent quadrats (i.e., two-, three-, or four rows, etc. at a time). A fortuitous juxtaposition between sample unit size and the average distance among insects on plant occurs at different intersections between the transect line length and pest density. This overlap provides this technique its remarkable precision, provided the user is competent. Of interest is the fact that the dynamically changing size of the sample units permits application of techniques that analyze the spatial pattern (See Ludwig and Reynolds 1988) of insects counts.

With LIS sampling similar principles apply, but here, the greater the fraction of rows along a transect line that are found with at least one pest the greater the indication that the spatial pattern is fine-grained. Conversely, the greater the number of 'zero' rows found along the transect line, the coarser the grain.

The second point follows from remembering that as the number of individual insect pests increase per sample unit, the greater the proportion of sample units infested with insects (Wilson and Room 1983). Practically speaking for the 2 x 9 scouting method, this means that the greater the number of infested plants per stop, the greater the chance that any given stop will be found with at least one plant occupied by *Lygus*. Thus, it is just as important to have a measure of how many stops are infested as it is to have estimates of the proportion of infested plants. This measure is provided for by the ratio of occupied stops to total stops examined. Most importantly, as this ratio approaches one, the more likely it is that the spatial pattern of the pest is fine-grained. The smaller the ratio the more coarse is the grain of the dispersion pattern of this pest.

The previous comments, point out a conclusion I have reached after using quadrat sampling schemes (stated here without proof, but supported by experience). The conclusion is that as pests approach their economic threshold, the spatial distribution of the insects in the field is always random, never clumped, and is fine-grained. (This is a debatable point. So, I caution here that users may find that these sampling schemes will cause them to revise many classical, economic thresholds currently recommended. I welcome comments from users on this issue.)

By using the several ecological principles presented in this article along with the quadrat based sampling schemes, I believe that an adequate assessment of the pest status of *Lygus* in cotton can be determined more reliably, more quickly and with fewer total samples than any other sampling plan currently available.

Summary

The straightforward process of randomly choosing the baseline origin, followed by the random placement of a perpendicular transect line on that baseline, is an appealing concept in row crops due to its simplicity. This procedure provides the randomization necessary to obtain unbiased sampling units, and thus unbiased estimates of row crop attributes like stand and plant bug numbers per acre. This feature is the strongest point to make for advocating the use of LIS in cotton. In this respect, I believe no other randomization method currently used in row crops to avoid bias can surpass LIS.

Clever adaptation of the LIS method can be applied to other attributes such as blooms or boll weevils per acre. However, I emphasize that while LIS is not difficult in its application for stand or plant bug counts with a drop cloth this is not universally true. As the size of the attribute of interest becomes smaller with respect to the size of the plants within a row, LIS can become a time consuming task. An example is sampling for the number of small Heliothine larvae feeding in squares of bolls. If one is willing to pay the price in time spent searching for small sized attributes, however, the precision of the LIS method is still quite remarkable.

An alternate method for sampling for plant bugs after canopy closure was also described. Much effort has gone into developing and adapting the concepts used in these two quadrat sampling plans. However, much remains to be done. For example, I still need to demonstrate how to calculate the chances of an incorrect decision for samples taken from different densities and economic thresholds of pests. This important task is near completion and is the planned topic of further papers. Yet, even without this information, I have found that the plans described here are useful and work in commercial fields where plant bug scouting is the concern. I hope others find them useful, and especially want to hear from anyone who is disappointed in their performance. The discovery of reasons why these plans were not suitable in these cases may lead to the development of better plans.

References

Anderson, D.R., J. L. Laake, B.R. Crain and K.P. Burnham. 1979. Guidelines for line transect sampling of biological populations. J. Wildl. Manage. 43: 70-78.

Kaiser, L. 1983. Unbiased estimation in line-intercept sampling. Biometrics 39: 965-976.

Lucas, H.A. and G.A.F. Seber. 1977. Estimating coverage and particle density using the line-intercept method. Biometrika 64: 618-622.

Ludwig, J.A. and J.F. Reynolds. 1988. Statistical ecology: A primer on methods and computing. John Wiley & Sons, New York. 337 pp.

McDonald, L. 1980. Line-intercept sampling for attributes other than coverage and density. J. Wildl. Manage. 44: 530-533.

McDonald, L. 1991. VIII. Line-intercept sampling for two-dimensional particles, *in* Sampling of Biological Populations and Resource Selection Studies, Workshop Notes. University of Wyoming, Laramie.

Pielou, E.C. 1977. Mathematical Ecology. Wiley Inter-Science, New York.

Willers, J. L., D. L. Boykin, J. M. Hardin, T. L. Wagner, R. L. Olson, and M. R. Williams. 1990a. A simulation study on the relationship between the abundance and spatial distribution of insects and selected sampling schemes. Proc. Applied Stat. Agric., Kansas State Univ., Manhattan.

Willers, J.L., R.L. Olson, M.R. Williams, and T.L. Wagner. 1990. Developing a Bayesian approach for estimating the proportion of cotton plants at risk to insect attack. Proc. Beltwide Cotton Conf., Natl. Cotton Council Am., Memphis, TN.

Willers, J.L., S.R. Yatham, M.R. Williams, and D.C. Akins. 1992. Utilization of the Line-intercept Method to Estimate the Coverage, Density, and Average Length of Row Skips in Cotton and Other Row Crops. Appl. Stat. in Agric., Proc. Appl. Stat. in Agric., Kansas State Univ., Manhattan, Kansas.

Williams, M.R., T.L. Wagner, J.L. Willers, and R.L. Olson. 1991. Scouting protocol for arthropod pests of cotton in the Midsouth. Miss. Agric. For. Exp. Stn. Bull. 977.

Williams, M.R., T.L. Wagner, and J.L. Willers. 1995. Revised protocol for scouting arthropod pests of cotton in the Midsouth. Miss. Agric. For. Exp. Tech. Bull. 206.

Wilson, L. T., W. L. Sterling, D. R. Rummel, and J. E. Devay. 1989. Quantitative Sampling Principles in Cotton IPM. *In* Integrated Pest Management Systems and Cotton Production (R. E. Frisbie, K. M. El-Zik, and L. T. Wilson, eds.), pp. 85-120. Wiley & Sons, New York.

Wilson, L. T. and P. M. Room. 1983. Clumping patterns of fruit and arthropods in cotton, with implications for binomial sampling. Environ. Entomol. 12: 50-54.

Table 1. Line-intercept sampling scaling factors for 38 *in* row cotton planted with an 8 row planter, using transect lines either 8 or 16 rows long, and a 3 or 5 *ft* long segment of row. These constants are used to convert field counts of stand or plant bugs to estimates per acre.

Length of Row Sampled	8 Rows Sampled	16 Rows Sampled
3 Feet	573.2	286.6
5 Feet	343.9	171.9