Sampling Crops for Residue Analysis

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The validity of any pesticide residue analysis hinges upon intelligent sample selection. Obtaining a truly representative sample, the prime requirement, calls for careful design of the entire experiment: establishing plots of adequate size, providing enough replicates, and taking care to assure uniform distribution of the pesticides. Careful collection, preparation, and analysis of samples can then yield all the sound data the experiment is capable of furnishing

THE VALIDITY and usefulness of a pesticide residue analysis hinge upon an intelligent and realistic approach to the problem of obtaining a reliable sample. If the sample taken is not representative of the commodity or of the plot from which it was obtained, all the careful and costly work put into the subsequent analysis will be wasted because the results will not be valid. It is important to remember that the collection of a representative sample is influenced by a number of variables which have to be taken into account before standard sampling procedures can be established. These variables include, among others: the source of a sample (which may range from a sparsely-covered rangeland to a banana plantation); the size of the raw commodity itself (which may vary from a grain of rice to a watermelon); and the method of applying the pesticide (which may vary from hand-dusting to aerial application).

Of critical importance in obtaining a representative crop sample is the design of the experiment. Such defects as diminutive plots, insufficient replicates, non-uniform application methods, and lack of attention to and understanding of the sampling problem are likely to result in analytical data of little or no value to anyone. Equally important, such data frequently give rise to misleading and erroneous conclusions.

As Gunther and Blinn (3) point out, many investigators are aware of the weaknesses of residue chemistry. However, few are aware that many of the discrepancies frequently reported by residue chemists are ascribable to failure to consider all of the controlling factors, or parameters, when planning the field experiment or making a crop-residue study. Of these factors, sampling of the crop is one of the most important.

Once the experiment has been properly designed, the highest quality of data that the experiment is capable of furnishing can be achieved only through an intelligently planned and uniform sampling method. The analysis of a large number of samples chosen at random is one means of evaluating an experimental plot, but this approach is tedious and expensive; however, a large number of samples will at least reflect the condition of an equal number of locations in the plot. The mean of such results will represent, to a greater or lesser degree, the average level of residue on the plot.

For practical reasons, there is a limit to the number of samples which can be taken from a single plot. Also, the amount of analytical work involved, in itself, makes the analysis of a large number of samples from every plot prohibitive. Therefore, in order that the level of residue present on a crop can be represented by a practical number of analytical results, certain fundamental sampling methods must be carefully followed in the selection of the gross sample and in the reduction of the gross sample to the analytical subsample.

Sampling methods are influenced, from crop to crop, by variations in preparing or processing the crop. Sweet corn, for example, is husked before it is consumed for food. Therefore, any residue remaining on the husk is removed before the corn reaches the table and is of no import. Strawberries, on the other hand, receive no treatment except rinsing in water prior to being served. In many instances, humans and livestock consume different portions of the same crop (e.g., cereal grains, sweet corn, and sugar beets, from which a portion of the crop is processed for human food and the remainder for animal fodder). In such cases, appropriate sampling of both portions is required.

Collection of the Gross Sample

In general, the gross sample should not only be representative of the treated plot or field of interest, but should also reflect the residue level of the crop as it reaches the ultimate consumer. Size, physical nature, and other properties of each unit part of the commodity will, of necessity, determine the quantity and treatment of the gross sample to be taken; also, the size and physical characteristics of the plot being sampled will need to be considered.

The area sampled may represent a small experimental plot designed to give useful performance data for a pesticide, as well as data on the level of residue on the crop; in other instances, it may represent a field, meadow, or pasture on which an actual crop is grown or used for grazing animals. Where the plot is small and various pesticides or dosages are applied on adjacent areas, it is desirable to avoid taking samples from the border of a plot because of the possibility of contamination from drift or spray overlaps. On the other hand, where the area to be sampled is an entire field, meadow, or pasture, it is desirable to take a sample that represents the average residue level. Therefore, a large gross sample should be selected in such a manner that it definitely reflects the crop variations in the entire area.

The responsibility for deciding whether samples taken should be selected randomly, systematically, or selectively must rest with a qualified person who is capable of recognizing and interpreting the importance and usefulness of the residue data obtained. Thus, in setting up sampling stations or sampling methods, it is necessary that consideration be given to all factors that control the residue distribution over the entire plot or area.

Consideration must also be given to sampling of control plots. The samples taken must, of course, be taken from untreated plots in which variation in quality and quantity of the crop closely parallel those in the treated area. In order to achieve this, it may be necessary to treat the control plot with another pesticide. If it

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is necessary to resort to this treatment, the analyst must be consulted (preferably before the treatment is made) so that due consideration can be given to the presence of contaminating or interfering substances in the control sample. In collecting samples from the control plot, the borders of the plot should be avoided because of the possibility of contamination due to drift from nearby treated areas. This is particularly true for small plots where buffer strips are small or nonexistent.

Some commodity units are very large (e.g. watermelons) and present a few problems in the collection of a gross sample and in reducing the gross sample to the analytical sample (normally about 2 pounds in weight). All large commodities should be subdivided or quartered about a symmetrical axis, or the entire unit may be reduced to small pieces before the subdivision is done. The subdivision and mixing of the subdivided parts must be done in a way that prevents appreciable loss or gain of seeds, fragments, or the pesticide residue. This is especially important when dealing with volatile or water-soluble pesticide residues. Throughout the steps of subdivision and rejection of the extra, discarded pieces (to achieve a smaller total sample portion), it is important to chop or macerate the crop in such a way that the retention or rejection of one of the largest unit pieces would not appreciably affect the residue result obtained.

In order to simplify consideration of suggested sampling procedures, the various commodities are classified into the following general or typical categories:

1. Large tree fruits (such as apples, avocados, citrus, and peaches).
2. Nut crops (such as almonds, chestnuts, pecans, and walnuts).
3. Small fruits (such as blueberries, currants, grapes, and cherries).
4. Legumes for food (such as beans, lima beans, peas, soybeans).
5. Legumes for forage (such as alfalfa, clovers, and vetch).
6. Grains for food (such as barley, corn, oats, and wheat).
7. Grain for forage (such as corn and sorghum forage).
8. Leafy vegetables (such as collards, kale, and leaf lettuce).
9. Head crops (such as cabbage, cauliflower, head lettuce, and celery).
10. Cucurbits or vine crops (such as cantaloupes, squash, and watermelons).
11. Perennial crops (such as asparagus, artichokes, and rhubarb).
12. Solanaceous fruits (such as eggplant, peppers, and tomatoes).
13. Root crops (such as beets, carrots, onions, potatoes, and radishes).

Field Sampling and Subsampling

Large Tree Fruits. There are a number of factors that influence the residue level on tree fruits at harvest time. These include: variations in spray penetration through tree foliage; differences in exposure of various portions of the tree and foliage to weather; and differences in the chemical and physical nature of the surface of the crop. Because of these factors, a sufficient number of fruits is taken to provide a gross sample of approximately 100 pounds. If the plot is small, it is necessary to sample from each tree in the plot. Should the plot be so large as to make sampling of each tree prohibitive, the samples are taken from trees at random but not from fewer than 16 trees in as many sampling stations. In any case, fruits are taken at random from all four quadrants of each tree sampled.

If the plot is to be sampled at several intervals during the growing season and again at harvest (in order to establish residue vs. time dissipation rates), a small plot cannot provide enough 100-pound samples without depleting the crop. In this case, a gross sample of 25 to 50 pounds is collected but this is done with the knowledge that the smaller sample may not be as representative as the normal large sample. In collecting the gross sample, two persons circle the tree in opposite directions and take samples from each quadrant of the tree in order to minimize individual biases. Care is taken to sample from the outer and inner portions of the tree, selecting the fruit in proportion to its apparent abundance in any area. The gross sample is reduced, by careful mixing and quartering, to approximately 25 pounds. The composite sample is placed in a kraft-paper or heavy plastic bag, or the equivalent, pending further reduction in sample size and subsampling.

It is not always convenient further to reduce the gross sample immediately, especially in the case of large fruits, because the individual fruit must be reduced in size before this can be done. However, where the gross sample can conveniently be subsampled immediately, it is desirable to do so—but with consideration for the utility of the resulting residue data. For instance, is it important or necessary to peel the fruits and discard the peel, or is it desirable to leave each fruit (or fruit segment) intact?

It is necessary to know the ultimate end use of the crop to make the proper decision. For example, apples, peaches, and similar crops are often eaten fresh with the peel on; they may also be peeled, processed, and canned or frozen. Obviously, where the whole fruits are to be consumed in the fresh state, the fruits or fruit segments are analyzed with the peel intact; in the event the fruits are to be peeled and/or processed, the peel is removed and the sample for analysis is taken from the peeled material. Avocados are eaten fresh but the peel is rarely consumed. The analysis of this crop without first removing the peel tends to give misleading residue results.

It is extremely easy to contaminate the pulp of certain fruits by contact with the peel during the peeling process. Thus, considerable care is necessary in peeling certain crops for analysis in order to avoid accidental contamination.

When it is not convenient or desirable to reduce the size of gross sample by subsampling, it is often possible to store the sample without freezing for several days or longer and to subdivide it further just prior to analysis. This is true for apples, oranges, grapefruit, and other crops that do not readily spoil. Crops that spoil readily, or are easily crushed, are subsampled and frozen or otherwise preserved soon after sampling.

Subsampling is accomplished by cutting each fruit into halves or pieces, mixing, and quartering the pieces until a 6-pound sample is obtained. If feasible, the 6-pound sample is divided into 3 equal (representative) portions and the resulting subsamples are placed in suitable containers, labeled with complete information as to plot identification and treatment history, quick frozen, and kept frozen until analysis. If 2-pound samples are not adequate, several 6-pound samples are taken.

Nut Crops. Most procedures applicable to tree fruits are applicable to nut crops. However, the gross sample can generally be labeled and stored until subsampling is convenient or necessary. At the time the 2-pound subsamples are prepared, they are placed in paper or plastic bags or other suitable containers, labeled with complete information as to plot identification and treatment history, and stored. Since only the nut meats are consumed, the hulls and shells are removed and discarded at any stage prior to analysis.

If the outer edible hulls are to be used for animal feed, it is desirable to analyze them for residues. In such cases, a composite, 6-pound sample is prepared and divided into three equal subsamples. The nut meats, upon removal from the hulls, are placed in suitable containers, labeled, and stored until analyzed. Unless an unripe crop is taken, it is not neces-

sary to keep the samples or subsamples frozen pending analysis.

Small Fruits. Procedures followed with large fruits are also generally applicable to small fruits. It is advisable for two persons to pick from opposite sides of the row, simultaneously. Sufficient fruits are taken, from 12 to 24 sampling stations in the plot, to make up a gross sample of at least 20 pounds (preferably 50 pounds). For strawberries, a minimum-sized sample is taken because 50 pounds not only depletes the plot appreciably but also is an expensive sample. Again, it is necessary to remember that a small gross sample may be less representative than a large one. As in other cases, the gross sample is reduced (immediately if possible) by careful mixing and quartering to approximately 6 pounds. The 6-pound sample is divided into 3 equal subsamples, placed in suitable containers, labeled with complete information as to plot identification and treatment history, frozen (if necessary), and kept unchanged until ready for analysis. Small fruits, generally, keep for only a short time at ambient temperatures.

Legumes For Food. With the legumes used for food (peas, snap beans, etc.), it is general practice to harvest the commodity used for human food and at a later date to make the vines into animal feed (ensilage or hay). In the event that mechanical harvesters are used to separate the peas from the vines, a number of grab samples are taken at uniform intervals until a gross sample of approximately 25 pounds is accumulated from the plot. If the crop is sampled by hand, two persons work together and select pods from opposite sides of the same vine, simultaneously. Pods are selected from the interior and exterior portions of the vine in proportion to their apparent abundance in those areas. A gross sample of 50 pounds of peas (including pods) or 25 pounds of snap beans is collected; it is reduced to a subsample of about 12 pounds in the case of peas, or 6 pounds in the case of snap beans, by careful mixing and quartering. Pea pods are rarely, if ever, eaten except where the pods are collected and ultimately fed to animals. Therefore, the gross sample of peas should be hulled before subsampling it. The 12- or 6-pound sample is then divided into three equal subsamples, placed in suitable containers, labeled, frozen (if necessary), and held unchanged until ready for analysis.

Legumes For Forage. On taking forage samples, it is important to keep in mind that the residue content of protected and unprotected areas is likely to vary markedly. For this reason "total harvest" is practiced in the sample selection areas. Generally, the sampling is conducted so as to get average representation rather than a measure of the maximum or minimum pesticide residue in or on the crop. Frequently, the pesticide is applied in a nonuniform fashion; therefore, a large area is sampled in a methodical or in a random manner, thus assuring a representative sample. Twenty-four random sampling stations are established and 1-pound samples are reaped from each station, selecting the samples in accordance with general practice. (The forage is cut to normal harvest height—usually 2 inches above the ground.) The 1-pound samples are cut (or chopped) into 1-inch lengths, combined, mixed, and reduced by quartering to a 6-pound sample. The 6-pound sample is divided into three equal subsamples which are properly labeled and held in frozen storage until ready for analysis. If the plot is large, it is desirable to take 2- or 3-pound samples (instead of 1-pound samples) from each of the 24 stations. It is necessary to exercise care to avoid loss of leaves or small branches in reaping and storing the sample.

Where pea and bean vines are to be used as fodder after the peas and beans have been harvested, similar sampling procedures are followed. Where peas are harvested mechanically, the vines and pods are adequately sampled by collecting 24 one-pound samples at random—or at uniform intervals—from the discharge chute of the "viner" in a manner that assures representation of the entire plot. The gross sample is mixed, quartered, subsampled, and stored to await analysis.

Grains For Food. Grains are used for food only when they are ripe; therefore, grains are generally sampled at harvest. Since mechanical harvesting is generally practiced, the 24 one-pound samples needed are collected from the discharge chute of the combine or harvester, uniformly spacing them over the entire plot. The one-pound samples are combined and mixed, and the resulting gross sample is reduced by quartering to approximately 6 pounds. The 6-pound sample is divided into three equal subsamples, placed in suitable airtight containers, labeled, and stored in a cool, dry place until ready for analysis. If there is any doubt about the stability, under these conditions, of the pesticide residue expected, the sample is frozen and stored at subzero temperatures.

Corn plots can be adequately sampled by collecting five or six mature, dry ears from each of 24 sampling stations in the field or plot. The husks are stripped off (kept for analysis, if desired) and the kernels are removed from the cob. The kernels are composited to make up the gross sample which is reduced to three subsamples. For soft, immature ears, the kernels are carefully cut from the cob, divided into subsamples, and stored at frozen-food temperatures.

Grain Forage. Grain crops used for forage are adequately sampled by following the procedure described for legumes for forage. Corn plants, sometimes used for silage or fodder after the corn has been harvested, can be adequately sampled by following the procedure for legumes for forage, or by taking 24 pound-sized samples from the "ensilage cutter" or "stalk cutter" in such a manner that they are uniformly spaced over the entire plot. The gross sample is reduced to 2-pound subsamples as are samples of legumes for forage. If they are green or wet, the final samples are stored in the frozen state; the same conditions apply if it is not known that the residues are stable, even if the subsamples are dry.

Leafy Vegetables. Depending upon the size of the plot, 12 to 24 sampling stations are planned and about one-half pound of foliage is collected at each station. In most cases, only the foliage normally used for food is included in the samples; any yellow leaves, weeds, or leaves lying on the ground are rejected. The 12 to 24 samples are composited and the resulting sample is reduced by careful mixing and quartering to a 6-pound sample. Since leafy crops are always washed before being eaten, the components in the 6-pound sample are washed thoroughly. After washing, the excess water is removed by draining and/or air-drying, the components are chopped into small pieces, and the resulting material is mixed well and divided into three equal portions. Each sample is placed in a suitable container, labeled with complete information as to plot identification and treatment history, frozen, and held in frozen storage until ready for analysis. If the analysis is started within three days after picking, the subsamples are stored in a dry place at a temperature of 34° to 40° F. for this period. The samples are not frozen or analyzed, however, unless they are properly washed.

Head Crops. The sampling procedures described by Van Middelem (5) for cabbages and celery are generally applicable to other crops in this category. However, 25 heads (rather than 35 or 40) from each plot comprise an adequate gross sample. They are trimmed to marketable condition

and the trimmings are discarded. The heads are quartered and alternate quarters are combined to produce the gross sample. The gross sample is then reduced in size by cutting each quarter into pieces about 0.5 to 1 inch in size, mixing well, and quartering to a 6-pound sample which in turn is mixed and divided into three equal subsamples. The subsamples are placed in suitable containers, labeled with complete information, frozen, and held in frozen storage until ready for analysis.

Cucurbits or Vine Crops. This category comprises, in general, the melon and squash families. The rinds and seeds of these crops, except for summer squash, are not used for food or commercial feed. Occasionally, however, livestock are fed culls or are allowed to "clean up" a field at the end of the season. Therefore, before the crop is analyzed for residue, it is necessary to determine whether or not the rind (or peel) and the seeds are to be removed and discarded. Summer squash are usually cooked with the rind and seeds intact; therefore, they are analyzed with these component parts included.

Twenty-four to 48 component units, depending on their size, are collected from the plot in such a manner that each row in the plot is similarly represented in the gross sample. Each component unit is washed thoroughly, allowed to drain, and air-dried. Each washed unit is cut into quarters, and the opposite quarters are selected for the gross sample. Unless it is to be included in the sample, the rind is pared from the flesh of each piece, with care to avoid accidental contamination. The flesh portions are chopped, and, if necessary, mixed and quartered until there is only 6 pounds left. The 6-pound sample is cut into small pieces by means of a blender, ricer, or other suitable equipment. The resulting sample is mixed well and three 2-pound subsamples of the subdivided pulp are placed in suitable containers, labeled, and kept in frozen storage until ready for analysis.

Crops to be used for animal feed are handled in the same way except that they should not be washed or peeled before quartering and subdividing.

Perennial Crops. Enough component units are collected from 12 to 24 sampling stations (depending on plot size) to make up a gross sample of approximately 25 pounds. Each component unit is trimmed and washed (as for market), chopped into small pieces, and combined to make the gross sample. The gross sample is well mixed and reduced by quartering to about 6 pounds. Individual pieces in the sample are chopped into 1-inch pieces and the 6-pound sample is mixed, divided into three equal subsamples, packaged, labeled, frozen, and kept in frozen storage.

Solanaceous Fruits. The procedure is similar to that for head crops. For the larger crops, such as eggplant, 25 individual units comprise an adequate sample. For the smaller crops, such as tomatoes and peppers, approximately 100 units or 25 pounds are adequate. The crops in this category are consumed without removing the peel and at times, the seeds. The fruits comprising the gross sample are washed, the stems are removed, and each fruit is cut into quarters. One quarter is taken from each fruit to produce the combined sample. The pieces in the sample are chopped into approximately 0.5- to 1-inch pieces and the sample is divided into three equal subsamples. Care is exercised to prevent any loss of fluids from the crops during cutting, chopping, or combining. Subsamples are put in impervious containers, labeled, frozen, and kept in frozen storage.

Root Crops. Residues on root crops (excluding foliage) are of importance only at harvest time. Except when using systemic pesticides, it is not necessary, in general, to consider sampling at various intervals throughout the growing season to obtain dissipation rates, especially if the residues result from soil treatment. Component units are collected from 12 to 24 stations (depending upon the size of the plot) to make up a gross sample of approximately 50 pounds, after trimming off the tops and roots (20 pounds may be adequate for small crops such as radishes and green bunching onions). The gross sample is reduced to approximately 25 pounds (10 pounds for small crops) by careful mixing and quartering. The individual units comprising the composite sample are washed free of dirt before the sample is further reduced in size. In the case of large crops (beets, carrots, potatoes, etc.), the individual units are cut or chopped into 0.5- to 1-inch pieces, and the sample is mixed and quartered to produce a sample weighing approximately 6 pounds. The smaller crops are reduced to a 6-pound sample by quartering without cutting or chopping each individual unit into pieces. The 6-pound sample is divided into three equal subsamples, packaged, labeled, frozen, and stored as are head crops and solanaceous fruits.

Sampling During Processing

Frequently, it is necessary to sample a crop being prepared for processing (canned or fresh frozen). The gross sample is best obtained at random from the cannery or frozen-food plant at a point in the operation subsequent to final washing or just prior to packaging. If individual treated and untreated plots are to be handled in this manner, three 4-pound samples are selected from some location in the processing plant, such as a conveyor belt, in such a manner as to obtain portions from the first, middle, and last of the material being processed from each plot. For control samples, six 4-pound samples are similarly selected from each untreated plot. By careful mixing and quartering, each 4-pound sample is reduced to a 2-pound subsample (three subsamples for the treated crop and six for the control crop). Each subsample is packaged and labeled immediately with complete identification information. Unless the sample is analyzed immediately, it is frozen and kept in frozen storage.

If it is necessary to use the same processing equipment for both the treated and untreated crops, it is essential that the untreated (control) crop be processed first to avoid possible contamination from any residues that may be present on the treated crop.

Storage and Shipment of Samples

Frequently, it is convenient or desirable to store the gross sample for a period of time before it is reduced to subsamples. The primary criterion in considering temporary storage is the ability of the crop to withstand spoilage or shrinkage. Potatoes, turnips, and root crops will keep in paper or plastic bags for several weeks without deterioration or appreciable loss of toxicant. (This practice is not used with certain toxicants that decompose on crops when not in the frozen state.) Other crops keep for several days without deterioration if the gross sample is placed in an impervious container and stored at 35° to 40° F.

Frequently, samples collected for residue analysis are shipped to some other location for analysis. Whenever possible, the shipment is made by air freight or air express. For samples that do not spoil (e.g. certain root crops, canned crops, etc.), surface transportation may be used. If a frozen sample is to be shipped, it is packed in a rigid, insulated container with sufficient dry ice to keep the sample frozen for at least 48 hours. The amount of dry ice needed varies somewhat, depending upon the container and the ambient temperatures. In warm weather, 2 pounds of dry ice per pound of sample is adequate to

keep samples frozen for a couple of days if a multiwall, corrugated paper container is used. Unless other arrangements have been made in advance, it is important that shipments be made in the early part of the week to avoid arrival at their destination on a weekend.

Certain types of samples can be shipped fresh without undue concern. For example, spinach, hay, certain fruits, and various other crops can be expected to arrive in good condition after being subsampled, packed in sealed paper or plastic bags, and shipped by air to their destination. Unless sampling and delivery to the laboratory can be accomplished within three days (maximum), however, this method of shipment is not attempted; instead, the samples are frozen and shipped with dry ice.

If it is necessary to ship large, heavy samples of crops, such as apples or potatoes, the individual samples are packed in such a manner as to eliminate any possibility of the sample containers' bursting, thereby permitting the component units to mingle and to lose moisture. Special precautions are always taken to prevent the possibility of check samples' being contaminated by mingling with treated samples, especially if it is necessary to pack both in the same outer container.

Finally, the full, correct address of the recipient is given to avoid any delay in routing or delivery. A delay of even one day sometimes leads to spoilage or loss of the sample. It is important, when making shipment by air, to notify the addressee of the shipment before the sample arrives, giving the details of shipment such as flight number and arrival time.

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