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Measuring Crop Density: Comparison of Volumetry and Stereological Methods

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For risk assessment and for registration purposes estimates of exposure to pesticides are essential. Models for re-entry exposure estimates have been developed over the past 10 years. The basic assumption of these models is that exposure results from the transfer of pesticide residues present at the crop during worker activities (Popendorf, 1985, Nigg et al., 1984; Zweig et al., 1985; Van Hemmen et al., 1995). Therefore, an estimation of the dislodgeable residue as a source strength for exposure is critical. The initial amount of the pesticide residue deposit will be affected by the use of the pesticide, i.e. formulation, application technique and application rate (Bates, 1990). Other factors determining the pesticide interception, thus the initial deposit, are the physical properties or characteristics of the treated crop itself e.g. the nature or geometry of the crop surface (Sundaram, 1991) and the foliar surface area. For chrysanthemums a relationship between pesticide interception and crop density is recently presented (Brouwer et al., 1994). Crop density is expressed by the leaf area index (LAI), i.e the surface area of the foliage per ground surface area. Present methods to estimate the LAI include stripping leaves from the plants and measure the surface area using a leaf area meter. To apply this meter all leaves must be positioned individually between a light source and the detector to measure the surface area. The leaf area meter uses the relationship between the decrease in intensity of transmitted light and the area of the leaves. For crops with small leaves, e.g. carnations, this procedure is very time consuming and furthermore destructive. Improving the measuring technique would overcome the obstacles to gather more data to estimate the pesticide residue. A new method to measure the crop density must fulfil some demands to be applicable: it should be practical, nondestructive; it should result in realistic values and be cheap in time costs and easy to use.

This paper reports the possibility to apply stereological methods to calculate the density of crop. Stereology is a set of simple and efficient methods for quantification of three-dimensional structures (Gundersen et al., 1988). These methods are mainly applied on light and electron microscopic images and are specifically tuned to provide reliable data from these two dimensional section of the structures. Two specific methods were investigated: the estimation of the volume fraction (the amount of crop volume per volume of space) and the estimation of the outer surface area of the crop. The results were compared to other known techniques to estimate these parameters.

MATERIALS AND METHODS

The first parameter investigated is the volume fraction (V_v), which can be calculated as follows:

$$V_v = V_c / V_t (m^3/m^3), \quad (1)$$

where

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V_c : volume of the crop
V_t : volume of the space in which the crop resides

The rational of the formula is as follows. Assuming a space Q comprising a volume V_Q and including a number of objects with total volume V_o then $V_V = V_o / V_Q$. In stereology it is well known (Gundersen et al., 1988) that V_V equals P_p, where P, is defined as the ratio between the number of points in space Q at which an object is situated (P_o) and the total number of points in the same space (P_Q). Inspecting all possible points in a space results in the absolute volume. Inspecting part of the points results in an estimation of the volume with an error. This error depends on the spacing of the points in relation to the density and size of the objects. Using trial and error an optimum can be found between the number of points to be tested and the error of the estimate. Locating the testpoints in space is the most difficult problem, which can be solved in a number of ways. Details on how this can be solved for the present investigation are given below.

The second parameter investigated is the surface density (S_V), which is an estimate for the outer surface area of the crop per volume of space in which the crop resides. The surface density of an object of arbitrary shape and orientation can be estimated via:

$$S_V = 2 * I_L \ (m^2/m^3). \qquad (2)$$

where

S_V : the surface density
I_L : ratio of the total number of intersections to the total length of test line

This I_L can be determined by counting the number of times any part of the crop touches a testline placed in the crops space. The placement of the testlines is discussed below. It should be noted that both stereological methods calculate parameters of the complete plants (stem and leaves) and not of the leaves alone as is used in the calculation of the LAI sofar. Using the stereologic estimates the presented relationship by Brouwer et al. (1994) could be redesigned or a LAI can be calculated. Presently only the second option is considered.
Since the S_V represents the complete plants and both sides of the leaves the LAI can then be calculated by:

$$LAI = S_V * L / 2 \ (m^2/m^3), \qquad (3)$$

where

LAI : Leaf area index
S_V : the surface density
L : Percentage of leaf surface area of the plant

The stereological methods are mainly applied on microscopical sections of tissue, so testpoints can easily be positioned on photographs of (sections of) the objects. Applying these methods for live crops, as proposed in this paper, forms a different situation which makes adjustments of the technique necessary. Carnation crop is used to demonstrate the technique. In this case testpoints are located using the iron grid, which is installed at several heights within the bedding to prevent the carnations to bend over or break. This grid consists of parallel and perpendicular iron wire spaced about 12.5 cm apart. The crossings form a nice grid which is used for systematic sampling.

Of course the plants grow higher then the top level grid. To estimate the density in that region a ruler is used which contains 21 marks spaced 2 cm apart. Randomly holding this ruler within the plants and counting the number of marks and the number of times any part of the plant hits a mark, gives a random sampling method to estimate the volume of the crop.
For the carnation crop the already mentioned iron wires of the grid are also used as a testline to count the number of intersections in order to calculate the surface area.
Plants will also grow at the sides of the bedding but are relocated inside the grid. Since the use of the two outer wires would thus lead to an overestimation of the volume they were not used.

The two stereologic parameters am validated by determining the real volume using a volumetric method described by Scherle (1970) and by calculating the surface area from this estimated volume.
The Scherle method to estimate the volume of the crop is based on the principle of Archimedes, which states that an object submerged in a liquid will loose weight quantitatively equal to the weight of the liquid displaced by this object. The loss of weight is caused by the upwards pressure which results in an equal increase of weight of the jar containing the liquid and the object. With the liquid being water, specific gravity = 1 g/cm^3 (at 4 °C), the increase in weight equals the volume of the object submerged.
For estimating the crop volume using this fluid displacement technique, the carnation crop is divided into three regions: top, middle and bottom. The top region corresponds with the region above the top-level grid and the bottom region corresponds with the lower 30 cm of the plants. Obviously the middle region is in between.
The leaves and stems of one sample of two rather different cultivars (Elsy and Princessa) are separated before measuring the volume to investigate the relative importance of these components.

From the volume data obtained with the Scherle method the surface area is calculated as follows. For the leaves the surface area of one side is calculated by dividing the volume by the thickness. From an investigation which established the relationship between the volume of leaves (measured with the fluid displacement technique) and the surface area of the same leaves (measured using the leaf area meter) the thickness of the leaves can be estimated. For carnation crop this relationship was: leaf area = 15.57 * leaf volume + 5.79 with a correlation coefficient 0.97 and an error of the estimate of the leaf area ≤ 9%. A thickness of the leaves of 0.06 mm on the average can be calculated from these results.
To facilitate the calculation of the surface area of the stems it is assumed that the stems have cylindrical shapes. It can be shown that the surface area of the stems can be estimated using the equation surface area = 4 * volume / diameter. From two cultivars the average diameter of the stems is estimated.

To measure the various parameters the following experiments have been conducted.
In five greenhouses the volume fraction of in total seven cultivars was measured.

* From the top-level grid crossings of all individual wires (except the two on the outside) in the length of the bedding were examined (at least 150 points per wire are examined).
* From the grids below the top-level crossings on as many wires as possible were examined. This counting is hampered by the density of the crop.
* In the area above the top-level grid, but below the top of the plants, a total number of 357 testpoints were examined using the ruler-method.

The cultivars examined were: Vanessa, Galida, Prestige, Elsy, Cortina Chanel, Silvery Pink, Pallas. All but the Vanessa and Pallas, which are standard carnations (with one central flower), are spray carnations (with a cluster of flowers).

In two greenhouses the volume fraction and surface density were estimated, where the volume was estimated by the fluid displacement technique for three different cultivars.

* The volume fraction was estimated using the top-level grid and the ruler method.
* The surface area was estimated using two wires of the top-level grid to locate in total 150 testlines.
* The crop is removed from the bedding and collected in three portions:
  - top region: all crop parts above the top-level grid;
  - middle region: all crop parts below the top-level grid and 30 cm above the ground;
  - lower region: all crop parts lower then 30 cm above the ground.

  For every cultivar three samples are taken.

The spay carnations examined are 2 years old White Giants and Elsy, and 1 year old Princessa each in triplo from two different greenhouses. Standard carnations were not evaluated.

The results were tested for significant differences using a t-test and for a relationship between parameters using linear regression analysis. Both test were performed using the statistical package SOLO (BMDP, California, USA).
The procedures used to measure the various parameters are described in detail in Table 1.

Table 1. Procedures to measure the various parameters are as follows.

1) The counting of grid crossings to estimate volume fraction:
* determine the grid crossing which are going to be examined,
* count the number of crossings;
* count the number of crossings which are hit by any part of the plant.

2) The counting of ruler-hits to estimate volume fraction:
* determine the crop volume which is going to be examined
* put the ruler into the volume with eyes closed,
* count the number of times any part of a plant hits a mark on the ruler.

3) The counting of testline hits to estimate the surface density:
* determine the gridlines which are going to be examined,
* count the number of lines;
* count the number of times any part of the plant touches a line.

4) Measurement of the volume by the displacement fluid technique:
* place a jar on a balance;
* insert an amount of water, enough to contain the parts of the crop to be measured;
* insert a holder made of fine thread or wire which holds the crop submerged in the water;
* record the total weight of the jar with the water;
* submerge holder including the crop in the water, take care that there is no direct contact between the holder and any part of the jar;
* record the total weight;
* the volume of the crop is the difference between the two weights recorded.

RESULTS AND DISCUSSION

The volumes estimated using the top-level grid method and the ruler-method are depicted in Table 2. For both methods the variance between persons is lower then between beddings (CV top-level grid: between persons 11% and between beddings 24%, CV ruler-method: persons 12% and beddings 19%). Therefore it is more efficient (i.e. results in a more reliable estimate) to count grid-points on more beddings then to use more persons to do the counting. Using an ascending number of points to calculate the volume fraction reveals that 500 points or higher lead to a variance lower than 10 % on the average.
For the number of hits on the individual wires in each cultivar, a two sample T-test revealed that for two cultivars (Silvery pink and Cortina Chanel in the same greenhouse) one wire at the same side of the bedding (at which the experimentor started counting) was significantly lower than all other wires ($p < 0.05$). In the other cultivars no differences between the wires could be demonstrated indicating that at that level the plants are evenly distributed.
There is no significant difference between the ratio top-level grid method: ruler-method of the cultivars (T-test; $p > 0.05$). Moreover, there is no significant relationship between the volume fraction estimated by the top-level grid method and by the ruler-method (regression analysis, $p > 0.05$

The density of the crop on grids at other heights then the top-level grid made the estimation very time consuming. So this was done only in three cases. The volume fraction increased from the top downwards until approximately 50% of the height of the crop and then stabilized. The increase in volume fraction depended on the cultivar investigated. From the bottom of the crop (at 20% of the total height) downwards, the volume decreased again.

Table 2. Mean volume fraction (V_v), coefficient of variation (% CV) and number of analysts (n) per cultivar is given at two regions within the plant: the height of the top-level grid and above this grid.

Carnation cultivar	Crop height (cm)	Top-level grid method				Ruler-method		
		grid-height	V_v	% CV	n	V_v	% CV	n
Vanessa*	160	120	7.6	18.6	3	5.1	14.0	3
Galinda	110	60	8.6	0.8	3	4.3	16.4	2
Prestige	90	30	7.5	11.0	2	4.3	10.6	2
Elsy	105	75	5.6	21.8	3	5.2	16.2	2
Cortina Chanel	90	47	4.3	4.8	2	4.7	25.4	2
Silvery Pink	100	65	4.6	3.2	2	4.4	13.6	2
Pallas*	155	105	4.0	26.4	3	3.5	7.3	3

*Vanessa and Pallas are standard cultivars; all others are spray cultivars.

The results of the various experiments to test the application of the parameter surface density are given in Table 3. One should keep in mind that normally the surface area is considered to be the total surface area of the objects. In this paper this area is divided by two to calculate a one-sided surface area in analogy with the previously applied LAI (Brouwer et al., 1994). Due to the low number of data no valid relationship could be established between V_v and S_v. The volume estimates do not appear to be different from the estimates given in Table 2.
Two parameters are determined using the method of Scherle: the ratio between volumes of leaves and stems and the total volume of the plant at the different regions. The results are given in Tables 4 and 5.

To calculate the outer surface of the crop from its volume the diameter of the stems must be known. Of two cultivars this diameter is measured at the three regions mentioned. No significant differences between the two cultivars were found, but the diameter from the bottom region significantly differed from the diameter of the other regions (t-test, $p < 0.05$). The mean diameters of the upper and bottom regions of the stem, 3.6 and 4.9 mm, respectively, were used in the calculations for the surface area. The results are given in Table 6. The data represent estimates for the outer surface area of one side of the leaves and the total surface area of the stems. The total surface area was only moderately dependent on the ratio between the volumes of leaves and stems.
With regard to the volume fraction the following can be concluded. Examining 500 crossings, spread over 5 or more beddings, will be sufficient to accurately estimate the volume fraction of the cultivars. Even though the ruler-method is less time consuming, it leads to more reliable results: testing in 5 beddings, at about 300 points each, leads to sufficiently accurate estimates of the volume fraction.
The ratio between the volume of the crop at the top-level grid and the volume above that grid is not different for the cultivars but too variable to be used as an estimator (Table 2).
In general, estimation of the volume fraction using data from the individual wires revealed no differences. This indicated that the volume fraction was not related to location within the width of the bedding.

The volume fraction seems to be constant between approximately 20 % and 50 % of the total height of the crop. From 50% upwards a linear decrease of density is observed, which depends on the cultivar. More data are needed to substantiate this conclusions.
The results indicate that the surface density can be determined more accurately than the volume fraction since the coefficient of variation is lower (Table 3). The data in Table 3

Table 3. Mean volume fraction (V_v), Surface density (S_v) and coefficient of variation (% CV) of three cultivars of carnation.

Carnation cultivar	V_v top-level grid		V_v ruler		S_v	
	mean	%CV	mean	%CV	mean	%CV
White Giant	11.7	3.8	3.3	21.0	45.8	3.2
Elsy	2.8	43.6	3.2	24.8	20.2	1.6
Princessa	3.0	43.3	6.0	9.1	21.6	1.1

Table 4. Percentage volume of leaves and stem of different regions of two cultivars of carnation.

Carnation cultivar	Lower region		Middle region		Top region	
	leaves	stem	leaves	stem	leaves	stem
Elsy	28	72	46	54	68	32
Princessa	41	59	61	39	60	40

Table 5. Mean volume fraction (V_v), coefficient of variation (% CV) and total volume in which the plants resides (dm^3) of plants at the different regions of three cultivars of carnation, estimated using the Scherle method.

Camation cultivar	V_v Lower region			V_v Middle region			V_v Top region		
	mean	%CV	Volume	mean	%CV	Volume	mean	%CV	Volume
White Giant	1.13	3.1	605	1.29	10.9	495	0.40	23.6	990
Elsy	1.02	13.6	385	1.14	7.7	550	0.41	9.8	495
Princessa	0.84	13.9	385	1.78	10.1	330	0.38	12.8	605

suggest that there is a relationship between the volume fraction using the top-level grid method and the surface density for carnations. The number of data need to be enlarged to confirm this.
The percentage leaves and stems of a plant depends on the region and the age of the plant (Table 4). At the bottom the volume of the stems is relatively large, whereas at the top the leaves have a larger volume. As expected from visual inspection, the volume of the leaves in the bottom region in older plants is less than in younger plants. Although these data are used for the calculation of the surface area of the total plant, they are of less importance, since a smaller volume of leaves is compensated by a higher volume of stems.
The trend in volumes per region calculated from the Scherle methods (Table 5) corresponds with the data of the volume fraction estimated using the counting method at several heights in the crop reported above (Table 2).

Table 7 compares the parameters for the different regions. There is a large discrepancy between the volume fraction obtained by the different methods. As stated before, the stereological methods are designed for use on photographs of sections of objects. In that situation the points are very small compared to the size of the object. In the present case the crossing of the wires are as large as most of the stems of the carnations. Moreover, the sampling grid is normally positioned upon an image of a situation, while in the experiments described here the grids purpose is to support the stems of the crop to prevent damage to the plants. In this way the grid interferes with the natural position of the crop, so the counting method may be biased and overestimate the real volume fraction. Since the amount of bias is not known the "real" volume fraction can not be estimated accurately using this technique.

Table 6. Surface area of the different regions of three carnation cultivars, calculated from the volume fraction estimated using the Scherle method.

Carnation cultivar	Surface area (m^2)		
	bottom	middle	top
White Giant	10.5	13.4	9.2
Elsy	5.5	12.2	4.8
Princessa	5.4	13.2	5.1

Table 7. Comparison of the different parameters of three cultivars of carnations.

Carnation cultivar	Volume fraction				Surface area		
	Top-level grid	Scherle middle	Ruler	Scherle top	S_v top-level grid	Scherle middle	Scherle total
White Giant	11.7	1.29	3.3	0.40	45.8	13.4	34.2
Elsy	3.5	1.78	3.8	0.38	20.2	12.2	23.4
Princessa	3.0	1.14	6.0	0.41	21.6	13.2	23.5

The volume fraction estimated with the ruler-method has no relationship with the volume fmction estimated with the top-level grid (Table 3). This is probably due to the fact that the ruler method is a sampling method which, in contrast to the grid sampling, does not interfere with the natural position of the crop, thus seems more reliable. Still the volume fraction is highly overestimated when compared to the volume measured using the Scherle method. Because the volume of the crop above the top-level grid is very low, the number of hits counted is very low. In fact this number is probably too low to lead to a valid estimation of the volume density.

With respect to the surface area estimation the dimension of the wires is not as important as for the estimation of the volume fraction, but the interference with the natural position remains, which again results in some overestimation of the surface area.
It seems obvious to compare the S_v of the top-level grid with the volume of the middle region, measured either with the counting method or the Scherle method. As mentioned above, the volume fraction was relatively high between 20 and 50% of the height of the plants and relatively low at lower and higher regions. The S_v of the top-level grid represents the larger portion of the crop. Therefore the S_v at the top-level grid is compared with the total volume of the crop as measured using the fluid displacement technique. This leads to the conclusion that

the S_v can be used to estimate the surface area of the plants. The LAI (of one-sided leaves) calculated using the surface density then ranges from
4 - 8 m^2/m^2 approximately. This value agrees with the LAI's calculated previously. The LAI for different flowers ranged from 3.5 (lily) to 7.5 (tulip) (unpublished data).

To summarize the conclusions. Due to the inability to create a sampling grid which fulfils the requirements of the stereologic methods an absolute volume fraction can not be estimated using the methods applied, although the relative measures give insight in the distribution of the plants in a bedding and the spread between the different cultivars.
In contrast the surface area can be estimated using the stereologic method. Since the worker comes into contact with leaves as well as with the plant the use of the surface area calculated from the surface density seems to be appropriate in the model to estimate the exposure. Also this method fulfils the demands mentioned before.

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