
PRESSURE-VOLUME CURVE ANALYSIS PROGRAM

USER'S GUIDE

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***** INTRODUCTION *****

This guide is a description of how to use the pressure volume (PV) curve analysis program for the IBM-PC (DOS environment) microcomputer. It is intended to assist the user in running the program as well as give some detail on program function.

The program was written almost 20 years ago and has not received much in the way of updating. Therefore the graphics capabilities are fairly crude. You might want to take advantage of the option for saving the results of the analysis and use some other graphics program for creating nice graphs.

Pressure-volume analysis has been used extensively in plant research for estimating the osmotic and pressure components of water potential in various plant parts as well as for providing information on bulk tissue elastic properties. The theory and methods of PV analysis are described by Tyree and Hammel (1972), Tyree and Richter (1981), Richter et al. (1980), and Wilson et al. (1979) to name a few.

The traditional approach has involved graphing the data points with the negative inverse water potential on the y-axis, and either the volume of water expressed from the xylem or the relative water content at the measured water potential on the x-axis. Generally a two-phase curve is produced, having a non-linear region at high water content and a linear region at low water content. A straight-edge is then used to help judge which points belong in the linear region of the curve. Thus determined, it is possible to obtain estimates of tissue osmotic potential at any water content. By subtracting the osmotic potential from the water potential at that water content, the bulk turgor pressure in the tissue can also be estimated.

The development of computer programs by the author for analyzing PV-curves arose out of a project involving over 200 sets of data. Aside from the obvious desire to reduce the time required for analysis, it was apparent that the traditional graphical approach involved a certain degree of subjectivity in determining the points to be included in the linear region. See the paper by Schulte and Hinckley (1985) for a description of the various approaches that were tried. All programs assumed that data were collected by the "weighing" method with saturated weight, dry weight, and the series of water potential/fresh weight pairs.

The earliest method involved applying simple linear regression to the linear region of the curve, but this method was quickly abandoned. The program included on this disk applies a non-linear least squares analysis, using the equations referred to as "PVC" in the Schulte and Hinckley (1985) paper.

When the PV-curve analysis program (PV) is run, the file name of a data set will be requested. The non-linear least squares fit uses the linearization or Taylor-series method (Draper and Smith, 1966). When the least squares routine has completed, a menu will be presented to allow for the selection of various output forms or further calculations.

This program is written with the Microsoft QuickBASIC system, and provided as a compiled, executable (.EXE) file. If you want to print any of the graphs on a dot matrix printer that is graphics-compatible (capable of using the "Prtsc" key), the DOS program called "GRAPHICS.COM" needs to be run (only once unless the computer is rebooted) before starting the PV program.

Math coprocessor support is provided automatically; the program will run with or without a coprocessor, although it will run several times faster if a coprocessor is present.

As a note to programmers, if the computer contains a math coprocessor and you wish for it to be ignored by this program, add the statement "NO87= " (one or more spaces after the equal sign) as a DOS environment variable. This may be done by typing "SET NO87= " at the DOS prompt. Coprocessor use may be restored by typing "SET NO87=" without a space after the equal sign. This DOS environment variable only affects coprocessor use by this program and has no hardware affect.

***** DATA ENTRY *****

The program reads data from a file that you may create with any editor or even a word processor if you make sure to save the file as a simple ASCII text file without word processor formatting. Follow the format shown below. The intended units are grams for all masses and megapascals (MPa) for all potentials.

"data set name"
saturated mass
dry mass
number of data points to follow
fresh mass,water potential
fresh mass,water potential
(and so forth)

Here's a sample data set...

"A"
.3462
.167
9
.346,-.18
.342,-.63
.333,-1.79
.328,-2.37
.319,-2.98
.317,-3.05
.312,-3.24
.298,-3.73
.285,-4.42

***** ANALYSIS OF PV-CURVE DATA *****

To start the PV-curve analysis program from the DOS prompt, simply type "PV". After a title banner and some other activities, the default parameter error tolerance is displayed and there is an opportunity to change it. A simple carriage return indicates acceptance of the displayed value. This error tolerance indicates to the non-linear least squares routine a maximum value that all parameter corrections may have before the iterations will cease. For a description of this iterative procedure, see any text on non-linear least squares analysis or Draper and Smith (1966). Decreasing the tolerance value below the default may provide slightly greater accuracy, but longer program running times will be required. Experience has suggested that tolerance values less than 0.01 generally will not provide greater accuracy in the results, given the limits in accuracy of the PV-curve data points themselves. Tolerance values of 0.1 may even be acceptable.

Next enter a file name or "?" for a directory of the disk. If the file is found, the data will be read into memory. The program will allow you to enter multiple data files in case you want to combine data sets. So after reading the data from a file, the program will keep asking for another file name until you just enter a carriage return (press enter) all by itself.

As the non-linear least squares procedure involves successive iterations to determine final values of the model parameters, initial values or guesses are requested. The displayed default values may be adequate, or in cases where convergence does not occur, these initial values may be changed.

When the initial values have been entered, the nonlinear estimation begins and the corrections to these parameter values will be indicated after each iteration. When all corrections become less than the parameter error tolerance value, the program will display a main menu. From this menu, the various outputs may be selected or the analysis may be re-run.

If convergence is not obtained...

Ideally, the displayed parameter corrections will rapidly approach zero, although some oscillation may be apparent. If this does not occur, the values may continue to oscillate forever (more than a dozen or so iterations). If the corrections are only slightly greater than the error

tolerance value, re-running the analysis with a larger tolerance may prove helpful (press ESC to get out of the iterations, see below). The most exciting failures-to-converge, however, occur with wild oscillations of the parameter corrections, a parameter such as the relative water content for turgor loss becoming negative, or a parameter approaching infinity!

First of all, it is important to realize that non-linear least squares algorithms are not like simple linear regression methods that quickly produce a clear, unambiguous response. Non-linear least squares involves an iterative, successive approximation procedure. As in simple linear regression, a minimum error fit is the goal, but the difficulty may be more apparent if you try to visualize an error surface like a topographic map, and you are searching for the minimum point. For some functions and data sets, the surface may have a single well-defined low point that is easy to find. Other situations may be complicated by multiple low points where you get lost in a "false minimum" or wander back and forth over a narrow ridge. In addition, consider that the surface really involves four dimensions in this program.

With that in mind, the most common reason for nonconvergence is that the data set does not have a linear region - either because of measurement errors or because the data collection was not carried out to a low enough water potential. Generally three points are required from water contents lower than the turgor loss point (TLP). In some cases, the plant tissue may have lost turgor at a relative water content (R) much higher than suggested by the default initial value (0.85). Sometimes increasing this value to 0.90 or even 0.95 will allow convergence to occur because a linear region will be more "apparent" below the TLP estimate.

In general, either try changing the initial values from the default settings or plot the data. The data can be plotted from within this program (from the main menu, select "DISPLAY GRAPH", and then "PV curve"), although if the iterative analysis has not completed, the fitted curve may not be meaningful. Occasionally a mis-entered or mismeasured data point is found in this way.

At any point in the program when input is not being requested from the keyboard, such as when a graph is being drawn on the screen or when the least squares iterations are running, pressing the ESC key stops the execution and returns the program to the main menu. This feature is not completely tested, so I'd like to know if it causes problems. If the program is requesting input, the carriage return must be pressed after the ESC key in order for it to work.

Error Messages

In order to minimize program length, the only error messages that this program generates are the "FILE NOT FOUND" message when a requested file name is not found on the disk, and the "DISK ERROR" message indicating that some type of disk reading error, such as the disk drive door being left open, has occurred. All other errors will cause the program to generate an error number that a programmer might find useful.

```
*****  
*****          PROGRAM OUTPUT          *****  
*****
```

When the non-linear least squares routine has completed successfully, a main menu appears from which various output options are available. Selecting "DISPLAY OUTPUT" gives final estimates of the parameter values along with some statistical information concerning model fit: R-squared, the sum of squares regression (SSR), and the sum of squares total (SST). Subsequently, the data points are displayed along with estimates based on the fitted equation and final values of the parameters.

Selecting "PRINT OUTPUT" also provides the information described above, together with original data points, to a printer.

Selecting "SAVE OUTPUT TO FILE" allows you to save all of the analysis results to a file. My intent was for this file to be a complete record of the analysis, and so it includes all the original data points, the fitted model parameters, and a table of variables that may be used by another program for creating graphs of the various water potential components, the elastic modulus and capacitance. A sample output file (actually, the one generated by running the analysis of the sample data set named "A" shown above) is shown below. The first section shows the input data file. Next comes a line with the fitted model parameters. Lastly a table is included with data that may be useful for making graphs.

Output file format:

Output file from PV (Version C) for: data set name

Input data file was:

```

saturated mass  dry mass  number of data points
point no.  fresh mass  1-R  water pot  -1/water pot
  -- repeat for all data points --
p-function coeff  (1-R)tlp  tlp Osm pot  sat Osm pot  sym-
plastic fraction
1-R  -1/water pot  water pot  osm pot  pressure  elas  capac
  -- repeat for (1-R) from 0.0 to 0.4 --

```

Abbreviations:

```

R - relative water content
pot - potential
tlp - turgor loss point
sat - saturated
osm - osmotic
elas - modulus of elasticity (MPa)
capac - hydraulic capacitance as change in volume
        divided by change in water potential (m3/MPa).

```

Sample output file:

Output file from PV (Version C) for: A

Input data file was:

```

.3462 .167 9
1 0.3460 0.001 -0.180 5.556
2 0.3420 0.023 -0.630 1.587
3 0.3330 0.074 -1.790 0.559
4 0.3280 0.102 -2.370 0.422
5 0.3190 0.152 -2.980 0.336
6 0.3170 0.163 -3.050 0.328
7 0.3120 0.191 -3.240 0.309
8 0.2980 0.269 -3.730 0.268
9 0.2850 0.342 -4.420 0.226
8.040 0.149 -2.983 -2.386 0.742
0.000 12.487 -0.080 -2.386 2.305 26.578 6.015E-09
0.004 5.060 -0.198 -2.398 2.201 25.634 6.182E-09
0.008 3.205 -0.312 -2.412 2.100 24.715 6.355E-09
0.012 2.362 -0.423 -2.425 2.001 23.837 6.529E-09
0.016 1.881 -0.532 -2.438 1.906 22.990 6.706E-09
  -- continues for another 94 lines --

```

Selecting "DISPLAY GRAPH" will allow various plots to be displayed on the screen. A menu of choices will appear including the standard PV-curve plot and other plots of pressure potential and the modulus of elasticity. After a particular plot is selected, the user is given the choice of manually selecting graph parameters (axis ranges and so forth) or letting the program automatically select reasonable values. The "manual" option also allows you to magnify small regions of plots. For example, selecting a narrow range for the x- and y-axis around the turgor loss point of the PV-curve will fill the graph with this portion of the curve. Some care should be used in selecting graph parameters; for example if the y-axis is to be numbered with

whole numbers (decimals = 0) and the range is 0 to 8, selecting 5 for the number of tic marks will produce an incorrect numbering of the axis.

The selection "PRINT GRAPH" should dump the last displayed graph to a graphics-compatible printer. This selection calls a DOS interrupt (5) similar to the "print screen" on the keyboard. In order for this routine to work, the DOS-supplied program, "GRAPHICS.COM" must be run before starting the PV programs. The effects of this program will be maintained until the computer is rebooted, so it need only be run once. The "GRAPHICS.COM" program is not much of a talker, and nothing appears on the screen when it is run.

The selection "WATER POTENTIAL COMPONENTS" will give estimates of water potential, osmotic potential, and pressure potential for any relative water content.

***** LITERATURE CITED *****

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