RACC
Research on Adaptation to Climate Change

REFERENCE MANUAL FOR HIGH SCHOOL TEAMS 2014-2015

INDEPENDENT

Vermont EPSCoR
CWDD
Center for Workforce Development & Diversity
AT SAINT MICHAEL'S COLLEGE

VT EPSCoR Research on Adaptation to Climate Change (RACC) in the Lake Champlain Basin High School Program 2014-15

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Introduction:

Established in 2011, the VT EPSCoR CWDD is one of two centers funded by the National Science Foundation and created through the Research on Adaptation to Climate Change in the Lake Champlain Basin (RACC) award. RACC is focused on understanding the effects of changing climate on the Lake Champlain Basin and to develop adaptive management strategies for the Basin.

RACC builds transdisciplinary teams of social and natural scientists to study the Lake Champlain Basin as a coupled human and natural system affected by climate change. We combine collections of data on physical processes, governance, and land use with complex systems modeling. Models will enable scenario testing to help Basin managers and policy makers investigate how adaptive management can be designed and implemented to respond to climate change.

CWDD increases the Vermont Science-Technology-Engineering-Math (STEM) workforce in size and diversity through multiple approaches:

- Inspire diverse high school students and undergraduates to enter STEM careers by involving them directly in RACC research. Support the professional development of high school and middle school teachers through involving them in RACC research.

- Match high school teams, undergraduates and middle school teachers with RACC social and natural scientists, who will act as research mentors.
- Target support for girls and underrepresented minorities, veterans, economically disadvantaged high school students, and students with disabilities.
- Involve students from Puerto Rico, New York, Maryland, Texas and other locations outside Vermont to bring a diverse pool of participants into the STEM pipeline.
- Cap off the year with at the VT EPSCoR Student Research Symposium where CWDD participants share research results and network with other STEM professionals.
- Support Native American and First Generation Vermont college students through scholarships to study STEM majors in Vermont.
- Enable the Governor's Institutes of Vermont (GIV) to reach out to every high school in Vermont with scholarships so that girls and economically disadvantaged students can attend the STEM summer institutes and Winter Weekends.
- Work with the Vermont Technology Council to connect undergraduates and small technology businesses that provide students with paid internships.

Research on Adaptation to Climate Change in the Lake Champlain Basin (RACC):

The RACC center is organized around an overarching theme with three research hypothesis driven questions, involving a diversity of scientists and engineers from academia and the private sector that are integrated with public and private stakeholders, undergraduates, middle school teachers, and high school students and teachers. They will study climate change-driven impacts on hydrological processes and nutrient transport in the lake basin (Questions 1 and 2), and develop ecosystem assessment scenarios and models to inform the work of policymakers (Question 3 and Integrated Assessment Model (IAModel)).

Overarching Question**:** How will the interaction of climate change and land use alter hydrological processes and nutrient transport from the landscape, internal processing and eutrophic state within the lake and what are the implications for adaptive management strategies?

Question 1: What is the relative importance of endogenous in-lake processes (e.g. internal loading, ice cover, hydrodynamics) versus exogenous to-lake processes (e.g. land use change, snow/rain timing, storm frequency and intensity, land management) to lake eutrophication and algal blooms?

Question 2: Which alternative stable states can emerge in the watershed and lake resulting from non-linear dynamics of climate drivers, lake basin processes, social behavior, and policy decisions?

Question 3: In the face of uncertainties about alternate climate change, land use and lake response scenarios, how can adaptive management interventions (e.g. regulation, incentives, treaties) be designed, valued and implemented in the multi-jurisdictional Lake Champlain Basin?

For more information visit: www.uvm.edu/~epscor

2014-2015 High School Program:

The CWDD supports high school teams interested in engaging in RACC research as either Independent Project teams or Streams Project teams. This year will be the sixth year of the VT EPSCoR Streams Project. Each year, the project changes to align with the needs of the overall research program. Independent Project teams work on non-stream related research projects.

Goal: Increase the number and diversity of high school students interested in STEM careers.

Objectives:

- Students and teachers experience active research;
- Students and teachers develop scientific field and lab knowledge and skills;
- Students make connections with college science faculty, programs, and campuses

Strategies:

- Train students and teachers in watershed ecology, climate change, systems thinking, and field and lab skills during residential training week.
- Task HS teams with collecting high quality data for the VT EPSCoR research project Research on Adaptation to Climate Change (RACC).
- Convene a Symposium for presentations of RACC research progress, an opportunity for students to experience presenting scientific research, and a venue for students to see where their efforts fit into the overall research program.

RACC High School Program 2014-2015

Manual Contents

About this manual:

- Become familiar with it at the outset of your participation.
- Use the "Team Project" section of the manual to keep track of your research
- Use this in conjunction with the RACC website (www.uvm.edu/epscor/highschool) which hosts a wealth of additional resources:
    - data analysis tutorials
    - mapping and site information
    - links to useful websites
    - presentation and symposium information

Email cwdd@smcvt.edu if you need assistance. Your message will be directed to the appropriate staff member.

High School Team Calendar – Independent Projects 2014-15

June 23-27	**Training Week**
July – winter	Identify a research question Collect data / conduct investigation ➢ ➢ ➢ ➢ ➢
December–March	**Project Presentation** ➢ Export data from websites, if needed: www.uvm.edu/epscor/redir/streamsprojectdata and/or other data sites, if applicable ➢ Analyze data ➢ Create a poster or PowerPoint presentation describing your research
February	Submit application for 2015-165 program, if applicable
April, date tbd	Present your research at the 2015 VT EPSCoR Student Research Symposium!

Data Analysis and Presentations

Contents

Data Analysis Overview

You should begin thinking about your preparing your poster or presentation for the VT EPSCoR Student Research Symposium in April as soon as possible. The basis of your poster or presentation will be an analysis of the data you have gathered during the past year and/or historical data (from the Streams Project online database, or other sources).

The Streams Project has created a **data analysis tutorial** to help guide you through the process of exploring and asking more in-depth analysis questions about your dataset. This should be your primary guide for beginning your data analysis, but the VT EPSCoR CWDD staff members are always available to help you along the way. Some modules are Streams Project –specific, while others are useful to anyone interested in analyzing data.

The tutorials can be found on the website here:

http://www.uvm.edu/~epscor/new02/?q=node/1027

The first link on the page that says "Complete Tutorial Series - All Modules" will open a PDF with all of the modules compiled into one document. The subsequent links are for accessing modules individually. The following is a list of the individual modules and what they cover:

- Module 1: What is science?
- Module 2: Understanding Streams Project Data
- Module 3: Refining and Retrieving Data
- Module 4: Data Exploration
- Module 5: Statistical Analysis
- Module 6: Summarizing Results and Drawing Conclusions

In this tutorial, statistical analysis is demonstrated using Microsoft Excel. Within each module, look for the "WATCH VIDEO" icon that looks like this:

These videos help you visualize a number of procedures outlined in the tutorial. **NOTE: To be able to watch the videos, download the QuickTime Player, if it is not already on your computer: http://www.apple.com/quicktime/download/

Viewing and Downloading Streams Project Data

To view or download data in the Streams Project's database, go to the following location website:

www.uvm.edu/epscor/redir/streamsprojectdata

Once you are at the web page:

1. Select the stream sites for which you'd like data. If you'd like data from multiple sites, hold down the "Ctrl" button in between selections. If you'd like data for all the streams sites, select the first stream site, hold down the "Shift" button, and the select the last stream site in the list.

2. Select the report that represents the type of data you are interested in under "Available Reports."

3. Select the date range for which you'd like data.

4. Once you've made these selections click the "Generate Report" button.

5. You can view the data available for these criteria on the webpage that appears. If you click on the heading of a data field in the table, a little box will pop up describing the data contained in that field.

6. To download the data seen here, click the "Export to Excel File" text above the table and save the file on your local computer.

An explanation of the data in the database, and a description of how to download data from this web page can also be found in **Module 3: Refining and Retrieving Data** of the Data Analysis Tutorial. The link to this module can be found here:

http://www.uvm.edu/~epscor/new02/?q=node/1027

Presenting Your Data: VT EPSCoR Student Research Symposium

All participants of the RACC High School program commit to presenting their research findings at the annual Vermont EPSCoR Student Research Symposium. A symposium is a great way for researchers to present and discuss their work and it provides an important channel for the exchange of information between researchers. At the Vermont EPSCoR Student Research Symposium, participants have the option to choose whether they present their research through a poster or an oral presentation. Both are great ways to share your work!

Posters versus Oral Presentations

Although it can be challenging to present a year's worth of work in 10 minutes, oral presentations can be a rewarding experience because you are the only one front of an audience whose attention you know you have. Oral presentations are brief and consequently the presentation must be clearly and succinctly presented.

Posters are a visual presentation of information that is understandable to the viewer without verbal explanation. Poster presenters have the opportunity to share their work with one person at a time, over an extended period of time. This allows the presenter to describe and discuss their research in greater detail than would be possible in an oral presentation to significantly more people, and allows for dialogue with poster viewers.

Posters

A research or academic poster provides a means of communicating your research at a conference or research symposium. Posters printed by Vermont EPSCoR are 3' x 4' (or 36'' x 48"), horizontally or vertically aligned. Upload your final poster file when registering for the symposium by the deadline announced in early March. The CWDD will print and set up your poster at the symposium.

How to Create a Poster Using PowerPoint

For many, this is the first time creating a research poster. Here are some tips for making an informative and attractive research poster:

1. Open PowerPoint
2. Click the 'Design' menu/tab at the top of the screen and select 'Page Setup'
    i. Change the dimensions of the slide from the default setting to: Width=48, Height=36 (for a horizontal poster), or Width=36, Height=48 (for a vertical poster). This is an important **FIRST** step – if you change the dimensions after putting content on the slide, you will have to re-format all text boxes, graphs, tables, photos, etc.
3. Critical poster elements:
    i. Title, Author(s) and affiliation(s)
    ii. Abstract/Summary (*optional*)
    iii. Introduction/Background: a brief but important overview to secure the viewer's attention
    iv. Materials and Methods: a brief description of the processes and procedures used, photos (*optional*) should be >300dpi
    v. Results: outcomes, findings and data displayed through text, tables, graphs, photos, etc.
        - Bulleted lists (rather than paragraphs) may help the reader understand the most important findings
        - Tables, graphs and photos should have captions. Graphs should have a legend, avoid 3-D graphs as they are hard to interpret
    vi. Discussion/Conclusions: summary or discussion of the significance and relevance of the results, identify possible future research
    vii. References
    viii. Acknowledgements
    ix. Please include the following text somewhere on the poster: Funding provided by NSF Grant EPS-1101317
4. Upload final poster file when registering for the symposium

Tips:

A. Use the "Designing Conference Posters" website to get ideas on poster layout and to download poster templates: http://colinpurrington.com/tips/academic/posterdesign
B. Choose a background and text color scheme. No need to go crazy: a white/light poster with black/dark text is often much easier to read than a multi-colored poster. Use cool/muted colors, solid colors, a color gradient, etc.

C. Lettering can make a difference in how easy-to-read your poster is. Here are some suggestions:
    - Title: at least 72 pt., bold preferred
    - Section Headings: at least 48 pt., bold preferred
    - Body Text: at least 24 pt.
    - Avoid using all capital letters
    - Use sans serif (Arial) for titles & headings
    - Use serif (Times New Roman) for body text
    - Use bulleted lists where possible instead of paragraphs
    - Use *italics* instead of underlining
    - White or light colored lettering is hard to read on a dark background when printed. Use black lettering instead on a light colored background
D. Logos: Do not forget to include the logos for the organization(s) that helped make the research possible!
    - Funding source: The National Science Foundation's (NSF) logo can be used by recipients of NSF support for the sole purpose of acknowledging that support: https://www.nsf.gov/policies/logos.jsp. Please include the following text somewhere on the poster: Funding provided by NSF Grant EPS-1101317
    - VT EPSCoR, RACC, CWDD and others if they were important contributors. Logos are available on the "Resources" website: http://www.uvm.edu/~epscor/new02/?q=node/900
    - Your school logo!

Example posters from the 2013 VT EPSCoR Student Research Symposium:

http://www.uvm.edu/~epscor/new02/?q=node/1285

Oral Presentations

A research talk provides a means of communicating your research at a conference or research symposium. Oral presentations at the VT EPSCoR Student Research Symposium are limited to 10 minutes: 8 minutes to present your research, 2 minutes for the audience to ask questions. Presenters often use the general rule of "1 slide per minute"; however the number of slides needed varies based on the complexity of the content of the slides. Upload your final PowerPoint file when registering for the symposium by the deadline announced in early March or bring the file to the symposium on a USB drive. The CWDD will provide the computer, screen, podium, microphone and laser pointer for your use.

Oral Presentation Structure (*suggested*):

- Title, Author(s), Affiliation (1 slide)
- Outline, *optional* (1 slide): overview of the structure of your talk, some speakers prefer to put this at the bottom of their title slide, audiences like predictability
- Introduction/Background
    - Motivation and problem statement (1-2 slides): Why should anyone care? Most researchers overestimate how much the audience knows about the problem they are addressing.
    - Related Work (0-1 slides)
    - Methods (1 slide): Cover quickly in short talks
- Results (4-6 slides): Present key results and key insights. This is the main body of the talk. Its structure varies greatly as a function of the research conducted. Do not superficially cover all results; cover key result well. Do not just present numbers; interpret them to give insights. Do not put up large tables of numbers as your audience will not have time to take in that much information at once.
- Discussion/Conclusions (1 slide): summary or discussion of the significance and relevance of the results, identify possible future research.
- References
- Acknowledgements
- Please include the following text somewhere on your slides: Funding provided by NSF Grant EPS-1101317

Logos: Do not forget to include the logos for the organization(s) that helped make the research possible!

- Funding source: The National Science Foundation's (NSF) logo can be used by recipients of NSF support for the sole purpose of acknowledging that support: https://www.nsf.gov/policies/logos.jsp. Please include the following text somewhere on your slides: Funding provided by NSF Grant EPS-1101317
- VT EPSCoR, RACC, CWDD and others if they were important contributors. Logos are available on the "Resources" website: http://www.uvm.edu/~epscor/new02/?q=node/900
- Your school logo!

Example posters from the 2013 VT EPSCoR Student Research Symposium:

http://www.uvm.edu/~epscor/new02/?q=node/1283

Resources

RACC High School Resources: http://www.uvm.edu/~epscor/new02/?q=node/900

- Includes links to datasets available online, including:

  Data and Data Analysis

  - VT Department of Environmental Conservation Lake Champlain Long Term Monitoring
  - VT Department of Environmental Conservation Volunteer Monitoring
  - USGS Stream Gauge Data
  - Vermont Water Quality Data
  - NOAA Quality Controlled Local Climatological Data
  - VT EPSCoR Data Analysis Tutorials
  - Data Analysis in Excel

- Helpful hints on posters and oral presentations

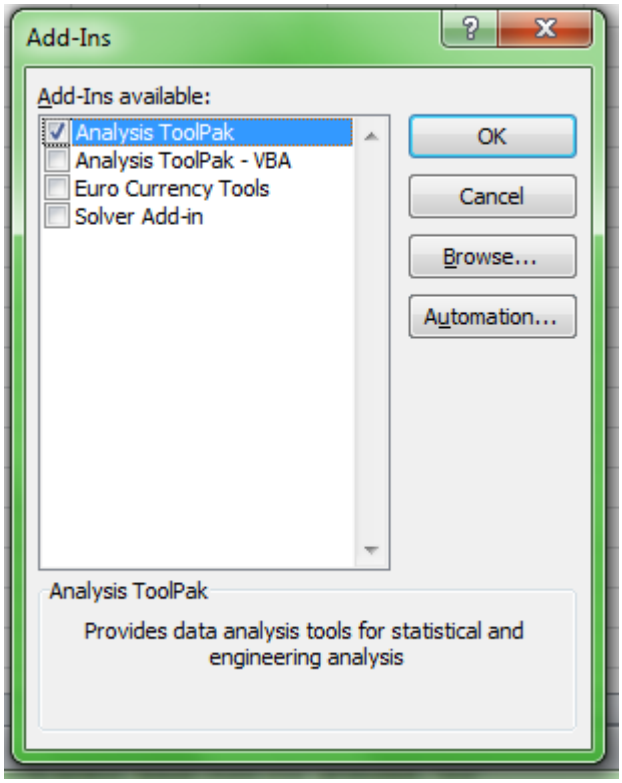
- High resolution logos to include on your poster, etc.

Data Webinar video by Dr. Declan McCabe:
http://www.uvm.edu/~epscor/new02/?q=node/1237

- Walks you through how to find different data sources online, how to groom and present your data using Excel, and how to use PowerPoint to create a presentation

Data analysis

Data analysis in Excel using Windows 7/Office 2010

- Open the "*Data"* tab in Excel
- If "*Data Analysis*" is not visible along the top toolbar then do the following:
    - Right click anywhere on the toolbar and select "*Customize quick access toolbar…*"
    - On the left click on "*Add-Ins*"
    - Near the bottom, use the pull-down menu and select "*Excel Add-Ins*" and click "*Go*" to bring up this menu:
    - 

    - Select the "*Analysis ToolPak*" and click "*OK*".

Using one-way ANOVA in MS Excel

Introduction: When your observations fall into two or more categories of continuous or even discrete variables, you may be interested in asking if the groups differ from each other. Is fish diversity higher in phosphorus-enriched ponds than in low-phosphorus ponds? Does the abundance of forest-floor plants differ between clear-cut, tornado-damaged, and control plots of forest? Questions of this nature are answered using analysis of variance (ANOVA). It is worth mentioning that in the case of 2 categories you can run a *t* test or an ANOVA and the result will be the same.

Analysis:

1. Organize your comparative data in adjacent columns (Table 1). There is no need to average them for analysis, and in fact averages will be calculated automatically during the ANOVA or *t* test.
2. From the "*Data*" tab, select "*data analysis*" (this must be added from the "addin" menu; see previous section).
3. Choose "*ANOVA single factor*"; click OK. Table 1 lists data from three habitats; so the *factor* of interest is habitat.

Number of mammal species		
island	mainland	peninsula
2	5	3
3	4	2
3	6	4
5	5	3
1	4	3
2	4	2

Table 1. Fake data for ANOVA

4. Click the tiny red arrow by "*input range*" and highlight all of the data including the column headings. Click the "Columns" button and check the "Labels in first row" box.
5. Select any of the output options that you like and hit "OK"
6. The output from the fake data should look like this:

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
island	6	16	2.666667	1.866667
mainland	6	28	4.666667	0.666667
peninsula	6	17	2.833333	0.566667

ANOVA

ce of Varic	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between (	14.77778	2	7.388889	7.150538	0.006593	3.68232
Within Gr	15.5	15	1.033333			
Total	30.27778	17				

7. The conclusion based on the *p-value* would be that number of species differ significantly among the three habitats. Note that the ANOVA does not tell you which groups are different, although in this case it looks like more species are found on the mainland and there is no difference between the island and the peninsula.
8. Finally, if you are making a comparison between just 2 groups, you can use exactly the same procedure. Or you could choose to run a *t*-test and it will give you a result that is mathematically identical to that produced by an ANOVA run on 2 groups. We could go back to the fake data and ask if the island and peninsula differ from each other by running the test without including the mainland data column.

Graphing ANOVA-type data: Use the averages to draw a bar graph. Add standard error bars to the graph. Calculate those using this formula: *=stdev(A1:A6)/Sqrt(6)* (assuming your data are in cells A1 through A6 and you have 6 data points). More detailed instructions are provided in the graphing section of this manual.

Regression in MS Excel

Does blood pressure increase with age? Does shrub cover decrease with increasing canopy cover? Is there a relationship between phosphorus concentration and algal cell density in ponds? All of these questions can be addressed using regression.

Nature of the data

All of the datasets described above are *continuous*; that is to say, they vary over some range without breaks. They are not *categorical* (like male and female), that are not *discrete* (like number of people in a single car; you would not typically think about 3.5 people in a car). As the range of a discrete variable increases (number of plants per hectare for example), the larger number means that what in fact is a discrete variable can be treated as continuous.

Graphing

We typically graph such datasets using a scatter plot (Figure 1) . If we have a basis for considering for example that running speed impacts heart rate, then we would use running speed on the horizontal (*x*) axis, and heart rate on the vertical (*y*). In this case running speed is the *independent variable*. The *dependent*, or *response variable* is heart rate because we expect it to *depend on,* or *respond to* running speed.

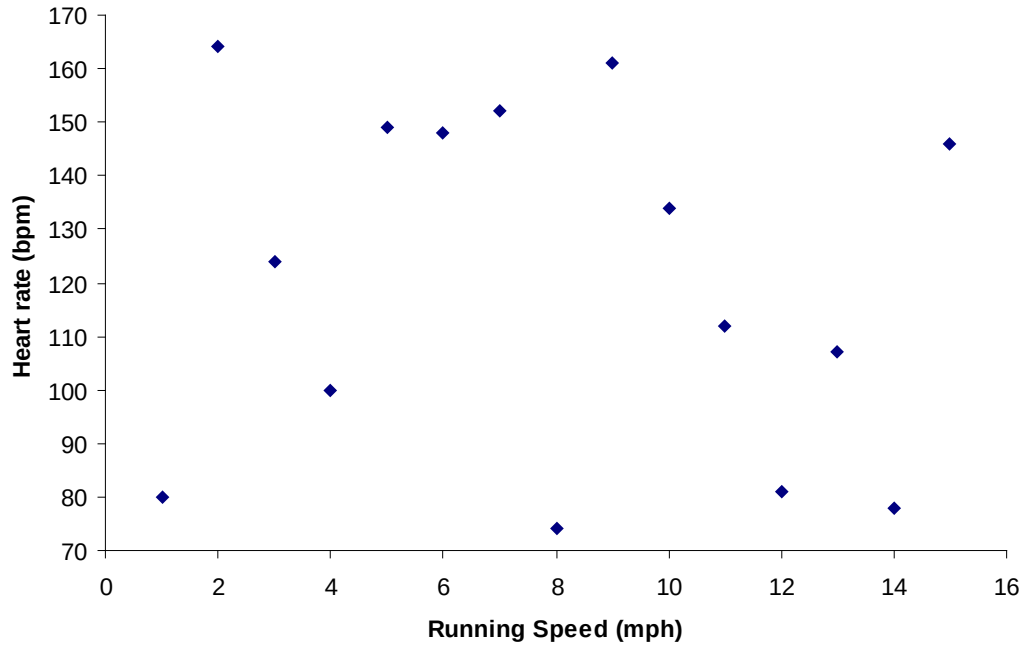
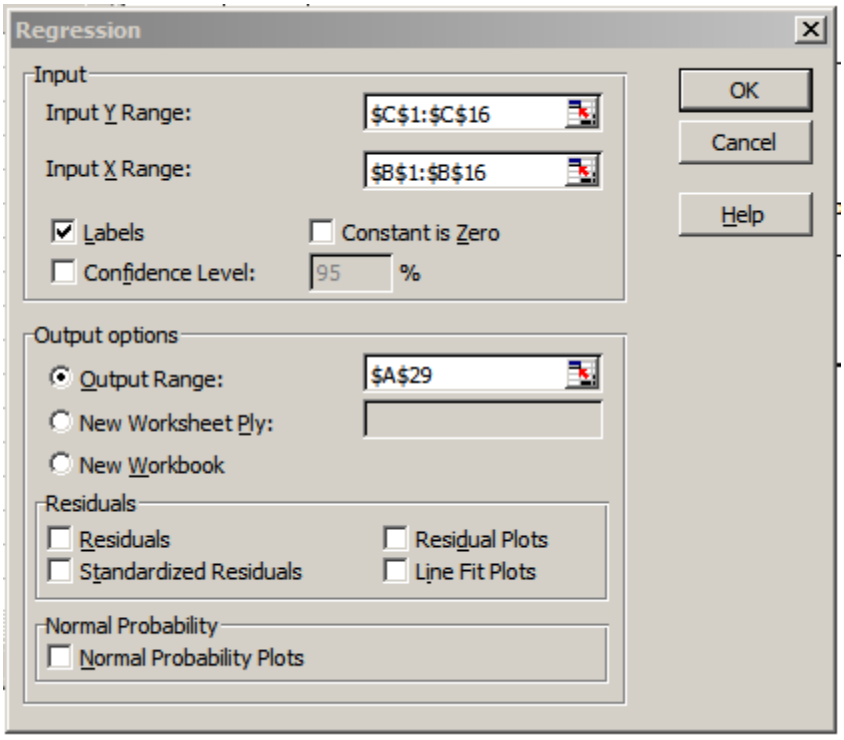


Figure 1. Fictional data representing the effects of running speed on heart rate.

Analysis: We might look at the pattern on the right and perceive a pattern, or not! As is the case with all statistics, the point is to remove subjectivity and have firm criteria for claiming a relationship. The analysis one would use for this sort of question is *regression*. There are many forms of regression for relationships of different shapes, but for our purposes we are considering only *linear regression*. In other words we are asking only if, and how well a straight line can describe the relationship between variables. In excel under the *Data tab,*select *data analysis, regression* to bring up this window:



The response variable goes in the Input Y Range and the independent variable goes in the Input X range. You can click on the tiny red arrow in each case and highlight the appropriate portion of the data (including labels). The output range simply is a place for the statistical output to go.

Output: Output from the preceding data set:

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.169583375
R Square	0.028758521
Adjusted R	-0.045952362
Standard E	33.23140781
Observatio	15

ANOVA

	df	*SS*	*MS*	*F*	*gnificance F*
Regressio	1	425.0892857	425.0893	0.384931	0.545699
Residual	13	14356.24405	1104.326		
Total	14	14781.33333			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0%*	*pper 95.0%*
Intercept	130.5238095	18.05655676	7.22861	6.66E-06	91.51499	169.5326	91.51499	169.5326
Running S	-1.232142857	1.985956467	-0.62043	0.545699	-5.52254	3.058255	-5.52254	3.058255

The number under *Significance F* is the *p* value. In this case the *p* value is greater than 0.05 and we can conclude that there is no relationship between running speed and heart rate.

Regression example 2: Along with other questions, Connon and Simberloff's (1978) paper examined the effect of sampling bias on collection data. They concluded that the number of collecting trips explained more of the variability in number of plant species observed on Galapagos Islands than did Island size or any other island feature measured. The data set:

Species	Collecting
57	10
31	6
3	1
25	4
2	1
18	6
10	6
8	1
2	1
96	13
94	12
40	7
5	2
54	13
346	27
47	7
2	1
102	10
108	9
12	6
69	10
290	28
237	24
440	38
61	11
283	29
45	6
16	3
21	5

And the statistical output:

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.973547
R Square	0.947795
Adjusted R	0.945861
Standard E	27.01902
Observatic	29

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	357850.2	357850.2	490.1875	7.62E-19
Residual	27	19710.73	730.0272		
Total	28	377561			

	Coefficients	*tandard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0%*	*pper 95.0%*
Intercept	-31.902	7.35061	-4.34005	0.000179	-46.9842	-16.8198	-46.9842	-16.8198
Collecting	11.61333	0.524536	22.14018	7.62E-19	10.53707	12.68959	10.53707	12.68959

Output	**Value**	**Standard interpretation**
p value	7.2 E-19	There is a very significant relationship between number of trips and number of species observed
Coefficient (of collecting trips)	11.61	The slope is positive telling us that as number trips increases, so does number of species seen. Negative slopes indicate the opposite trend.
R square	0.947	This measures how tight or strong the relationship is. In this case we can say that collecting trips explain 94.7% of the variability in number of species observed.

Graphing example 2: Connor and Simberloff's (1978) data set is presented graphically in the manual section on graphing. Compare how the data follow a tight linear pattern compared to the fake data on heart rate in this section.

Graphing

Figures in Community Ecology

All graphs, maps, photographs, and sketches are considered "Figures" and appear in a numbered sequence in the order cited in your paper. Any set of numbers and/or letters is considered a table and tables have their own numbered sequence (IE, even after three figures, your first table is still *Table 1*).

A good graph minimizes clutter and unnecessary 'ink'. Use the MS Excel "Scatter Plot" option to make graphs displaying continuous data on the vertical and horizontal axis. The species area data for the upcoming lab report are a good example; area on the *X* axis; number of species on the *Y* axis. **Remove** all of the following items added by Microsoft excel: "Series 1"; background color; frames on right and top; grid lines; 3D effects.

Scatter plots

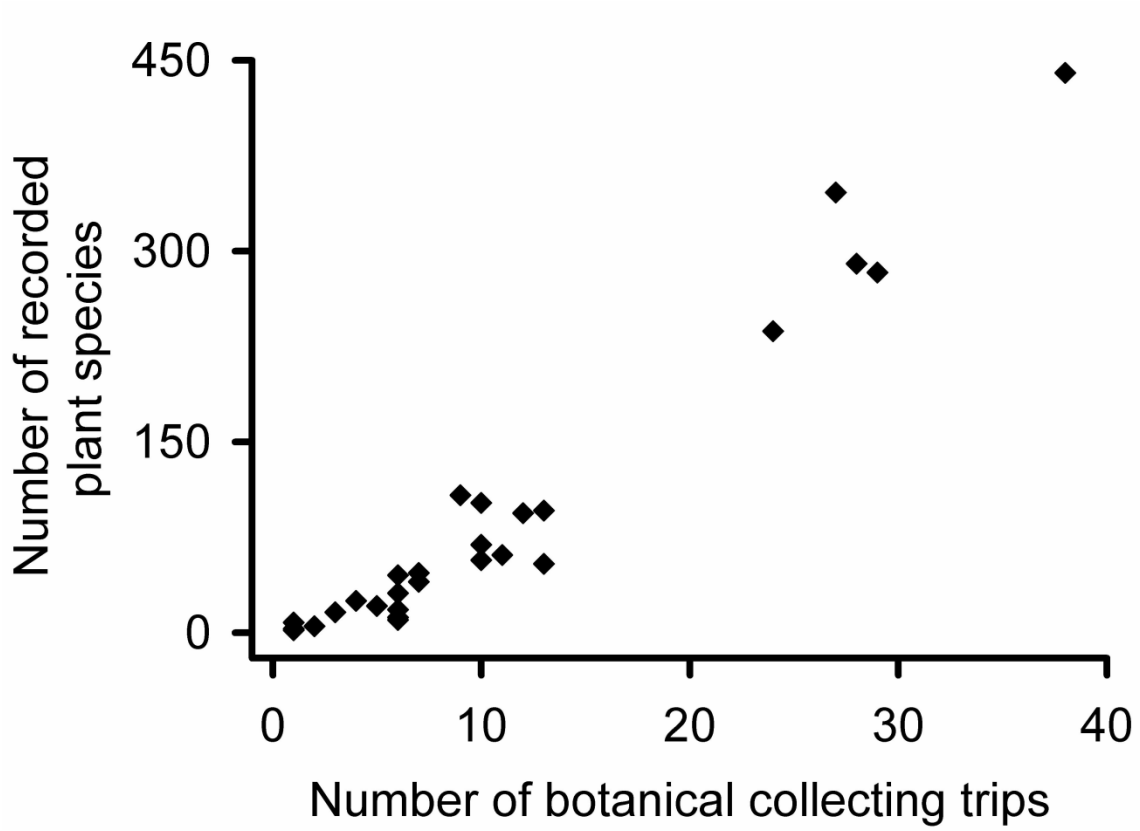


Figure 1. Illustrating the point that more sampling leads to more species observed. Connor & Simberloff (1978) analyzed data from collecting trips to the Galapagos Islands and found that number of collecting trips better explained number of species recorded than did island area, elevation, or isolation. Data extracted from Table 3 in Connor & Simberloff (1978).

The figure legend is always placed underneath and contains roughly a paragraph of information describing the figure content in sufficient detail that the figure stands alone. The

legend inserted by MS excel is useful *only if two or more data sets are displayed* on one graph using symbols.

This figure contains data that span the nearly entire range presented. If we were presenting data from only the largest five islands we would adjust the horizontal axis to run from 20 to 40, and the vertical axis from 150 to 450. Note that the axis lines have been thickened and fonts enlarged beyond the default. **Important**: Graphs should not start at zero, zero if the data range fall between 75 and 85 (for example).

Bar graphs

We use bar graphs when presenting the averages of *continuous* variables (on the *Y* axis) from one or more *categories* on the horizontal axis.

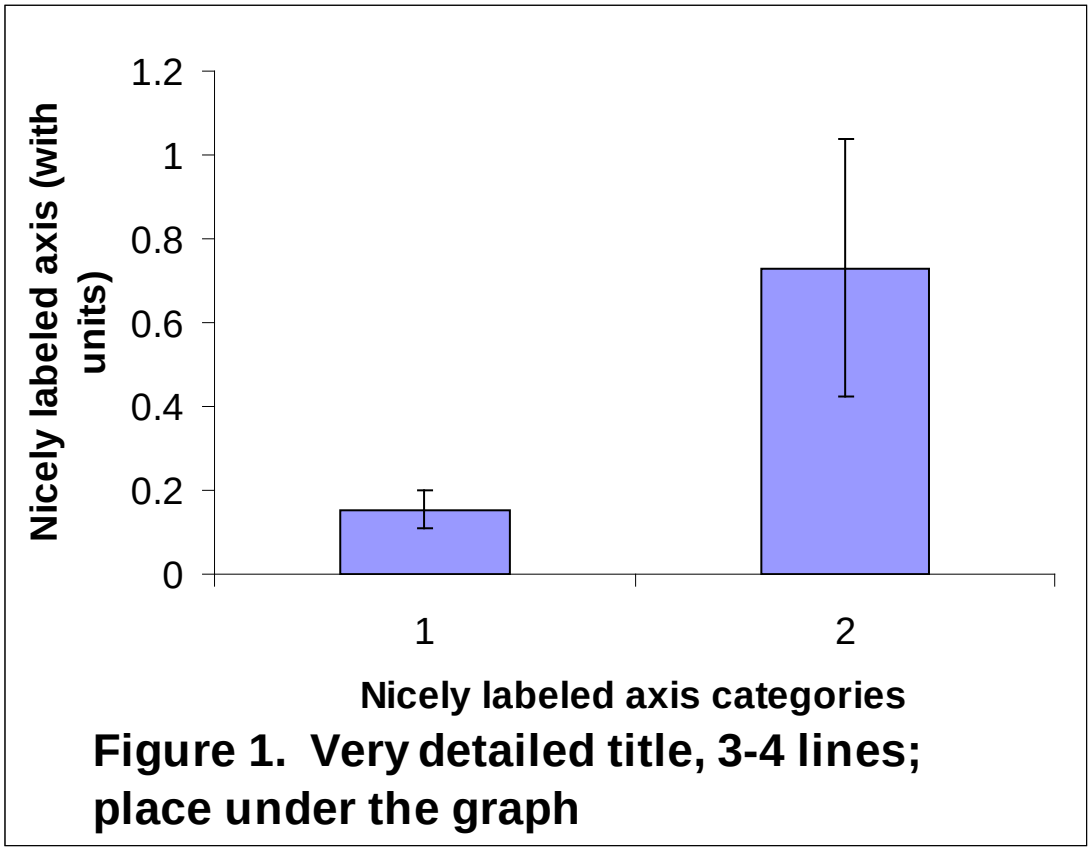


Figure 1. Very detailed title, 3-4 lines; place under the graph

The bar height equals the average of the response variables for treatments 1, and treatments 2. The error bars above and below the average in this case equal standard error; calculate these values as: (standard deviation)/(square root of the number of samples). The scale is appropriate to the data; if the averages were 150 and 200, I might start the axis at 100 rather than zero. **Important:** You should replace the numbers on the horizontal axis with names of sites or treatments (see example under adding error bars handout).

Adding error bars to bar graphs in excel

Introduction: Bar graphs are among the most common ways to present the averages of a set of treatments or conditions in community ecology and many other fields. Every average is based on raw data measured from a sample of several individuals. If I care about grass density in my lawn I might count the number of stems from several small quadrats and then calculate the average number of stems. The numbers of stems in each of my individual quadrats will be greater than or less than the average. In other words *there is variability in the raw data*. We might expect more variability in the heights of people than in the heights of Volkswagens. *Some data sets are more variable than others*. We use error bars above and below the average to depict that variability

How to measure variability: There are several metrics used to express variability. Standard deviation expresses the *variability in your sample* and is calculated in MS Excel using this Formula 1.

= stdev(A1:A6)…….Formula 1

The formula calculates the standard deviation from the raw data you entered in the cells *A1* through *A6* in the spreadsheet. You can refer to any set of cells in the spreadsheet by changing the letters and numbers in parentheses in Formula 1. The disadvantage of standard deviation is that it increases in magnitude as your sample size decreases. Samples can be expensive or time consuming to collect and so we often need to work with small sample sizes. What we really need is a measure of variability in the entire population, and not just in our sample.

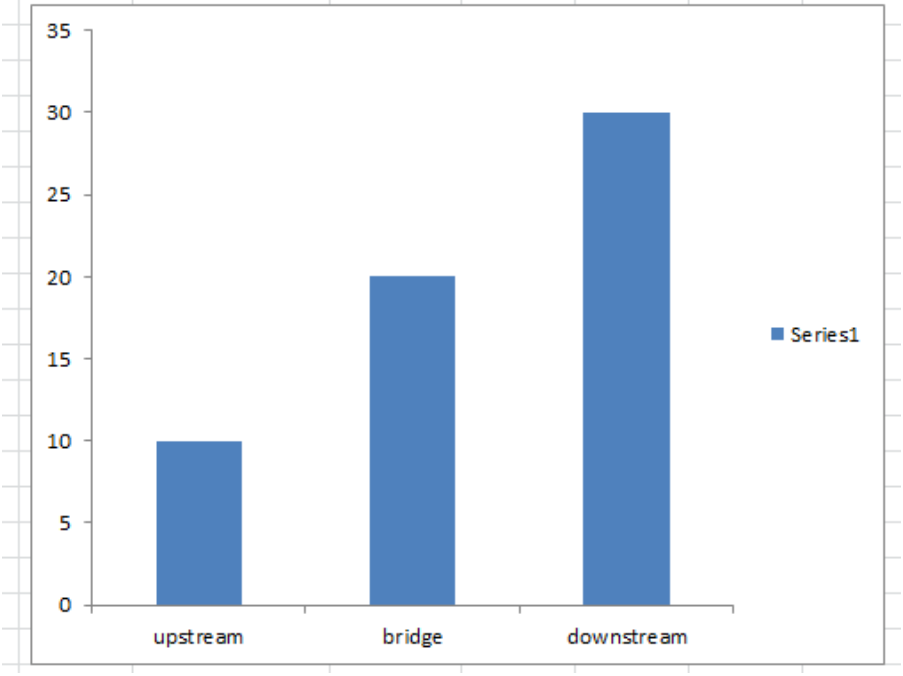
Standard error adjusts the value of standard deviation based upon the sample size using Formula 2

= stdev(A1:A6)/sqrt(n)…….Formula 1

Where *n* = the number of replicates in your sample; don't enter the letter *n*, enter the number of samples you took or refer to a cell in the spreadsheet that contains that information. *Sqrt* calculates the square root of whatever value you use to replace *n* in Formula 2. **Standard error will be the preferred measure of variability used throughout this course**.

How to add the error bars to your bar graph:
Lay your data out as illustrated below. In this case the fake data represent the average number of insect species found several samples taken from each of three locations in a stream.

	upstream	bridge	downstream
average richness	10	20	30
standard error	2	4	6

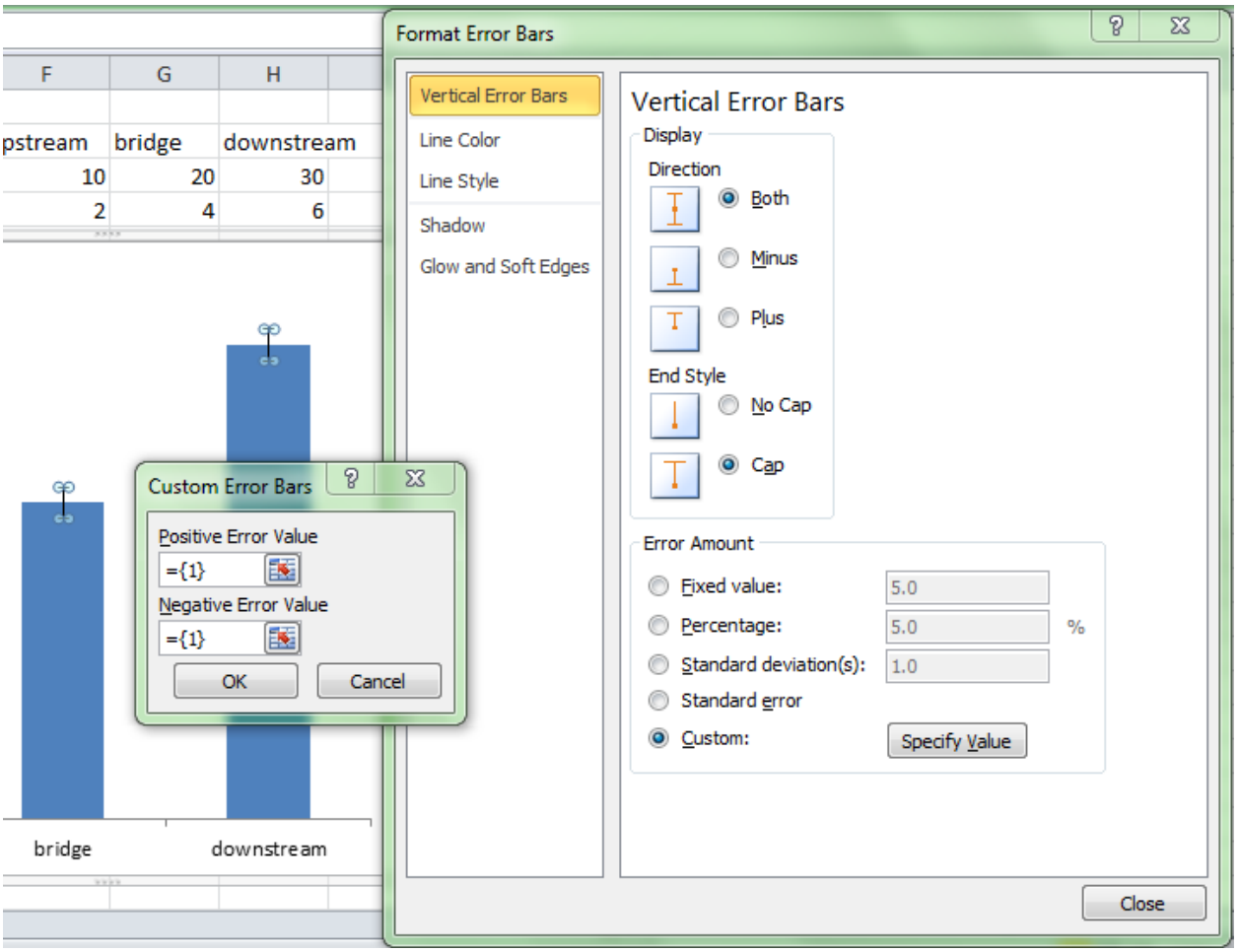


Note:

- Standard error values are underneath the graphed averages.
- The graph has been moved in the spreadsheet so as not hide the numerical values.

1. Click anywhere on the chart - this will reveal the "*Chart Tools*" at the top of the window. Click "*Layout*"
2. Right click on any bar in the graph – 2 small windows will pop up – work in the smaller upper one. Click the little drop down arrow and select the data set to which you'd like to add error bars (*Series 1* unless you have renamed the data set).
3. Now, go up to "*Chart Tools*" at the top and select "*Error Bars*"/ "*More error Bar Options*" (because all of the other options offered are, to be perfectly honest, fake).

4. Click "*Custom*" and "*Specify Value*".



5. Next click the tiny red arrow in the box under "*Positive Error Bar*"; highlight the values for the standard errors that are lined up under the averages. Hit "*Enter*"!
6. Now, you would think that having selected "both", that both the upper and lower error bars would be displayed; you would be wrong! Repeat the process for "*Negative Error Bars*".
7. Click "*Close*".
8. Truly beauteous error bars will now grace your bar graph!

Field Safety

Contents

First Aid Kit

When working in the field, it is important to be prepared for emergencies. Although you will not be traveling far from your car when you visit your field sites for the VT Streams Project, accidents may still happen. Therefore, a well-stocked first aid kit is an important thing to have. Carry a first aid kit with you to your site or keep one in the car. You may purchase a pre-made kit at the store, or you may make your own using the recommended list of items below as a reference. Whichever you chose, it is important to include any personal items such as medications and emergency phone numbers. Check the kit regularly and replace any used or out-of-date items.

- Adhesive bandages (assorted sizes)
- Antibiotic ointment
- Antiseptic wipes
- Instant cold compress
- Hydrocortisone ointment
- Scissors
- Sterile gauze pads (assorted sizes)
- Butterfly bandages
- Tweezers
- Prescription medications (asthma inhalers, Epipen)
- Emergency phone numbers
- Charged cell phone

Didmyo Fact Sheet

Didymosphenia geminate, commonly known as "Rock Snot" or "Didymo," is an aggressive freshwater alga that has undergone a recent large expansion in range. It has the potential to form nuisance blooms during which it can form mats several inches thick by attaching itself to streambeds by stalks that form a thick brown mat on rocks, plants, and other aquatic surfaces. The thick growth reduces the quantity and quality of aquatic habitat.

Didymo was detected in rivers of Vermont, New York, and New Hampshire during the summers of 2006 and 2007. Because the factors that cause Didymo to undergo rapid growth are unknown and there is no known method of eradication, it is important to prevent the spread of these algae to uninhabited streams. Therefore, ***we disinfect all waders and equipment when traveling between streams***. In order to prevent the spread of didymo to other regions waders should not be transported and used in different regions or countries.

Follow the link for a detailed description of Didymo by the Vermont Department of Environment Conservation Water Quality Division:

http://www.anr.state.vt.us/dec//waterq/lakes/htm/ans/lp_didymo.htm#how_can_I_disinfect

Disinfecting Waders

We have supplied your team with concentrated Quaternary Ammonium Disinfectant (Quat solution) to kill and prevent the spread of nuisance biological agents such as Didymo. This procedure is adapted from the Vermont Agency of Natural Resources method for equipment disinfection.

ATTENTION: Quat is a highly basic solution. Protective gloves MUST be worn when handling the concentrated solution. Once diluted with water, it is safe to handle

To prepare a 2.5% solution:

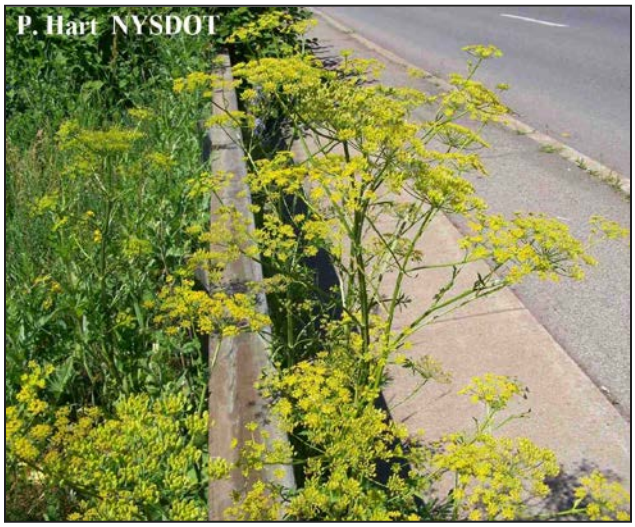
- Add 25mL of concentrated Quat to a spray bottle. Dilute to 1L. (For 500mL of solution, add 12.5mL of concentrated Quat and dilute with water to 500mL.) **Quat solutions should be replaced every 2 – 3 days to remain effective, so prepare only as much as is necessary for a site visit.**

- Fill the second spray bottle with water.

- When exiting the stream following sampling, spray waders and other equipment thoroughly with the 2.5% Quat solution. Let sit for ~2 minutes. Spray with the water to rinse.

Field Precautions

Poison Parsnip



- **Location**: Predominately found on the sides of highways and fields throughout Vermont.

- **Appearance**: The plants typically grow 3-6 feet tall and resemble Queen Anne's Lace, but the flowers are yellow instead of white.

- **Danger**:
  - The plant contains a high concentration of furocoumarin chemicals
  - The plant's juices may be transferred to your skin if you brush against the flower tops or broken leaves or stems
  - When the juices on the skin are exposed to ultraviolet light on both sunny and cloudy days the furocoumarin chemicals bind with nuclear DNA and cell membranes.
  - **This process destroys cells and skin tissue, causing severe burns in which the skin to reddens and blisters**

- **Protecting Yourself:**
  - Avoid exposure to the plant by choosing stream sites or access areas free from poison parsnip
  - If unavoidable, wear long sleeve shirts, pants (or your waders!), and gloves to prevent direct contact with your skin
  - Rinse and wash all clothing items and skin surfaces immediately following possible exposure. Keep exposed skin out of sunlight.

Poison Ivy

Poison ivy in spring.

Image © Jonathan Sachs 2002

Avoid poison ivy

Preventing contact with poison ivy

•Do not touch or handle any part of the plant

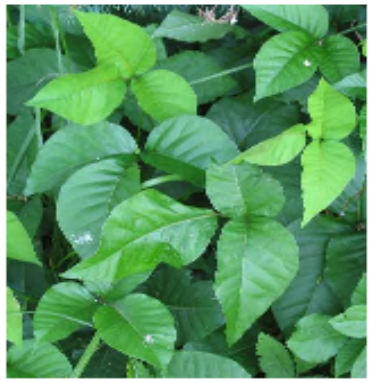
•Remove and wash shoes or clothing that has contacted poison ivy. Wash your hands immediately with soap and water

Preventative treatment Modified From http://poisoncontrol.uchc.edu

•If you have touched poison ivy, avoid spreading the oils to other body parts and wash the affected skin with soap and water within 15 minutes

•Use a nail brush to clean under finger nails

•Swab with rubbing alcohol after washing



Poison ivy in summer.

www.kentuckycrosswords.com

Myths Vs Facts: Fact #1: this fact list is modified from www.zanfel.com

Myth: Scratching poison ivy blisters will spread the rash.
Fact: Fluids from blisters will not spread the rash. Before blisters form, the rash can only be spread by unbound urushiol. Scratching of blisters can cause bacterial infection.

Myth: Poison ivy rash is "contagious."
Fact: The rash is a reaction to urushiol. The rash cannot pass from person to person after the urushiol binds to skin.

Myth: After the first time, I can't get poison ivy again.
Fact: Not everyone reacts to poison ivy upon first or subsequent exposures, people generally become more sensitized with each contact and may react more severely to subsequent exposures.

Myth: Once allergic, always allergic to poison ivy.
Fact: A person's sensitivity changes over time, even from season to season. People who were sensitive to poison ivy as children may not be allergic as adults.

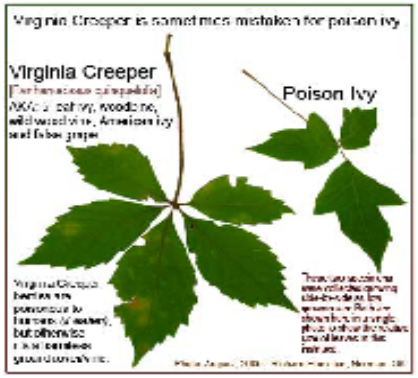
Myth: Dead poison ivy plants are no longer toxic.
Fact: Urushiol remains active for up to five years. Never handle dead plants that look like poison ivy without proper protection.

Myth: Burning is the best way to dispose of poison ivy.
Fact: The toxic oils from poison ivy spread in the smoke and can cause full-body rash and more serious health problems if inhaled.

Zanfel Laboratories provides poison ivy treatment brochures for free to BSA troops. Call 1800 401 4002

If a rash develops From http://poisoncontrol.uchc.edu

•Apply calamine lotion, cool compresses, or over the counter corticosteroid creams to lessen itching. Oatmeal baths can also help. Avoid scratching and cover open blisters to avoid infection. If face or genitals are involved, see a doctor for evaluation. If symptoms are persistent after these treatments see a doctor.



Ticks & Lyme Disease

T I C K S & L Y M E D I S E A S E

What Is Lyme Disease?

Lyme disease is a bacterial infection caused by the bite of an infected deer tick. Untreated, the disease can cause a number of health problems. Patients treated with antibiotics in the early stage of the infection usually recover rapidly and completely.

Where Is Lyme Disease Found?

In the United States, infected ticks can be found in the north-east, including New York State; in the upper Midwest; and along the northwest coast.

What Are the Symptoms of Lyme Disease?

The early symptoms of Lyme disease may be mild and easily missed. If you find a tick attached to your skin, remove the tick with tweezers and watch for the symptoms of Lyme disease. In 60-80% of cases the first symptom is a rash, known as *erythema migrans,* that:

- Occurs at or near the site of the tick bite.
- Is a "bulls-eye" circular patch or solid red patch that grows larger.
- Appears between three days and one month after the tick bite.
- Has a diameter of two to six inches.
- Lasts for about three to five weeks.
- May or may not be warm to the touch.
- Is usually not painful or itchy.
- Sometimes multiple rashes appear.

How Can I Safely Remove a Tick?

If you DO find a tick attached to your skin, do not panic. Not all ticks are infected, and your risk of Lyme disease is greatly reduced if the tick is removed within the first 36 hours.

To remove a tick:

- Use a pair of pointed tweezers to grasp the tick by the head or mouth parts right where they enter the skin. DO NOT grasp the tick by the body.
- Pull firmly and steadily outward. DO NOT jerk or twist the tick.
- Place the tick in a small container of rubbing alcohol to kill it.
- Clean the bite wound with rubbing alcohol or hydrogen peroxide.
- Monitor the site of the bite for the next 30 days, for the appearance of a rash. If you develop a rash or flu-like symptoms, contact your health care provider immediately.

What Else Can Be Done?

- Keep lawns mowed and edges trimmed.
- Clear brush, leaf litter and tall grass around the house, and at the edges of gardens and stone walls.
- Stack woodpiles neatly away from the house and preferably off the ground.
- Clear all leaf litter (including the remains of perennials) out of the garden in the fall.
- Keep the ground under bird feeders clean so as not to attract small animals.
- Locate children's swing sets and other play equipment in sunny, dry areas of the yard, away from the woods.

For more information on Lyme disease, contact your local health department or refer to the NYS Department of Health web site at ***www.health.state.ny.us***

- Do NOT apply repellents directly to children. Apply to your own hands and then put it on the child.
- When applying repellents, avoid the child's face and hands.
- Do not apply repellents on skin damaged by sunburn, cuts, bruises or other conditions, such as psoriasis.
- Avoid prolonged and excessive use of DEET.
- Do NOT apply repellents in enclosed areas.
- Do NOT apply directly on your face.
- Do NOT apply near eyes, nose or mouth.
- Wash treated skin and clothing after returning indoors.
- If you believe you or a child is having an adverse reaction to a repellent containing DEET, wash the treated area immediately and contact your local health care provider or local poison control center.

Also consider these important facts:

- If you tuck pants into socks and shirts into pants, be aware that ticks will climb upward to hidden areas of the head and neck, so spot-check clothes frequently.
- Clothes can be sprayed with DEET or treated with permethrin. Follow label instructions carefully.
- Upon returning home, clothes can be put in a high temperature dryer for 20 minutes to kill any unseen ticks. A shower and shampoo may help to dislodge crawling ticks, but this is not always effective.
- Any contact with vegetation, even playing in the yard, can result in exposure to ticks. Frequent tick checks should be followed by a whole-body examination and tick removal each night. This is the single most effective method for prevention of Lyme disease.

Ticks will attach themselves anywhere including the thighs, groin, trunk, armpits and behind the ears. If you are infected, the rash may be found in one of these areas.

Around the time the rash appears, other symptoms, such as joint pain, chills, fever and fatigue can occur, but they may seem too mild to require medical attention. As Lyme disease progresses, severe fatigue, a stiff aching neck, and tingling or numbness in the arms and legs, or facial paralysis can occur.

The most severe symptoms of Lyme disease may not appear until weeks, months or years after the tick bite. These can include severe headaches, painful arthritis, swelling of the joints, and heart and central nervous system problems.

How Is Lyme Disease Diagnosed?

If you think you have Lyme disease, you should see your health care provider immediately. Early diagnosis of Lyme disease should be made on the basis of symptoms and history of possible exposure to ticks. Blood tests may give false negative results if performed in the first month after the tick bite.

How Is Lyme Disease Treated?

Early treatment of Lyme disease involves antibiotics and almost always results in a full cure. However, the chances of a complete cure decrease if treatment is delayed.

In a small number of cases, Lyme disease can become a chronic condition. However, some patients have reported slow improvement and even an end to symptoms, months or even years after treatment.

How Can I Protect Against Ticks and Prevent Lyme Disease?

Deer ticks live in shady, moist areas at ground level. They will cling to tall grass, brush and shrubs, usually no more than 18-24 inches off the ground. They also live in lawns and gardens, especially at the edges of woods and around old stone walls.

Deer ticks cannot jump or fly, and do not drop onto passing people or animals. They get on humans and animals only by direct contact. Once a tick gets on the skin, it generally climbs upward until it reaches a protected area.

In tick-infested areas, your best protection is to avoid contact with soil, leaf litter and vegetation. However, if you garden, hike, camp, hunt, work, or otherwise spend time in the outdoors, you can still protect yourself:

- **Wear light-colored clothing** with a tight weave to spot ticks easily.
- **Wear enclosed shoes, long pants and a long-sleeved shirt.** Tuck pant legs into socks or boots and shirt into pants.
- **Check clothes and any exposed skin frequently** for ticks while outdoors.
- **Consider using insect repellent.**
- **Stay on cleared, well-traveled trails. Avoid contacting vegetation.**
- **Avoid sitting directly on the ground or on stone walls.**
- **Keep long hair tied back,** especially when gardening.
- **Do a final, full-body tick check at the end of the day** (also check children and pets), and remove ticks promptly.

What Do Ticks Look Like?

Two common types of ticks are dog ticks and deer ticks. Deer ticks can carry Lyme disease. Dog ticks can carry Rocky Mountain spotted fever but have not been known to carry Lyme disease.

Enlarged View Female Deer Tick

Female deer ticks have four pairs of legs and are red and black in color, while the male is all black. Young deer ticks - nymphs, are brown, the size of poppy seeds and very difficult to spot. An adult deer tick is only about the size of a sesame seed – still very small.

Deer Ticks Actual Size

Dog ticks are the most common type of tick, and, while feeding, can be as large as a small pea. They have four pairs of legs, are reddish-brown and are easier to spot. Dog ticks turn gray while feeding. Ticks can be found throughout the year, but they are most active during the spring, early summer and fall, when it is warm and moist.

Enlarged View, Male and Female Dog Ticks

Adult Dog Tick Actual Size

What About Insect Repellent?

Two active ingredients found in repellents are DEET (the label may say N, N-diethyl-m-toluamide) and permethrin. Permethrin is only used on clothes. DEET repellents or products come in many different concentrations, with percentages as low as five percent or as high as 100 percent. In general, the higher the concentration the higher the protection, but the risk of negative health effects goes up too. Use the lowest concentration that you think will provide the protection you need. The New York State Health Department recommends taking these precautions when using repellents that contain these active ingredients:

- Store out of the reach of children and read all instructions on the label before applying.
- Do NOT allow children to apply repellents themselves.

Cyanobacteria

What is cyanobacteria?
Cyanobacteria, also known as blue-green algae, are naturally occurring bacteria that are present in Lake Champlain and other water bodies around the world. Like plants, they use photosynthesis to convert sunlight into energy. Usually cyanobacteria cannot be seen by the naked eye. However, under certain conditions, the algae grow prolifically and are visible as blooms. The blooms appear as a cloudy pea green accumulation in the water. Generally, these blooms of cyanobacteria occur when there is a balance of certain factors including: an abundance of available nutrients, warm surface water temperatures, and calm winds.

Why should be concerned?
Unfortunately, certain types of blue-green algae produce toxins or poisons. When the algae die and break down, these toxins are released into the water. Exposure to these toxins have health impacts on humans and animals. Human health effects from cyanobacteria blooms vary depending on the type and duration of exposure (including inhalation of water droplets). In the summers of 1999 and 2000, the deaths of several dogs were linked to the cyanobacteria in Lake Champlain.

Photo source: Lake Champlain Basin Program

Identification and Avoidance: ***When in Doubt, Stay Out***
In general, blooms have the appearance of:

- Cloudy water as thick as pea soup or green paint on the water
- While generally green or blue-green in color, they can be brown or even purple
- A thick mat or foam may form as it accumulates onto shore

Blooms usually occur in August or September and can appear and disappear rapidly. There is no accurate way to identify the algae without a microscope. If you are suspicious, simply stay out of and away from the water.

References and Resources:
Check Current Conditions Online:
http://healthvermont.gov/enviro/bg_algae/weekly_status.aspx

Vermont Department of Health's Blue-Green Algae Guidance Document:
http://healthvermont.gov/enviro/bg_algae/documents/BGA_guide.pdf

Websites:
http://healthvermont.gov/enviro/bg_algae/bgalgae.aspx
http://www.lcbp.org/water-environment/human-health/cyanobacteria/

http://www.lakechamplaincommittee.org/lcc-at-work/algae-in-lake/

Photo Galleries:

http://www.lcbp.org/2012/12/photo-gallery-2008-cyanobacteria-blooms/
http://healthvermont.gov/enviro/bg_algae/photos.aspx#bg

Report a Blue-green Algae Bloom:
If you have questions or want to report a suspected bloom:
Call 1-800-439-8550 or 802-863-7220, or
email AHS.VDHBlueGreenAlgae@state.vt.us

If you believe that someone has become ill because of exposure to blue-green algae, seek medical attention and contact the Health Department at 1-800-439-8550.

Measuring Infiltration Rates

This exercise is included in the manual for RACC teachers to use with their classes, if interested. It is not a required data collection task for your participation in the RACC Streams Project and these data will not be uploaded to the Streams Project database.

Introduction:
Infiltration is the movement of water into a soil profile. The rate at which infiltration occurs is controlled both by the inherent properties of the soil and by the ways in which humans have modified the landscape. Infiltration rates, in turn, control runoff rates and soil erosion, which are important because these processes influence the behavior of hillslopes. This exercise is designed to introduce you to a simple method for measuring infiltration rates. You will use a ring infiltrometer to measure infiltration at plots that represent differences in disturbance of the soil surface. You may also measure the soil bulk density and gravimetric moisture content at the measurement sites and compare these to measured infiltration rates.

Methods:
Select two sites for measurement of soil properties and infiltration rates representing (1) a forested site showing no signs of noticeable compaction or human traffic, and (2) a site located on a designated hiking trail or one showing noticeable signs of compaction. You will extract soil cores from a location immediately adjacent to your infiltration test.

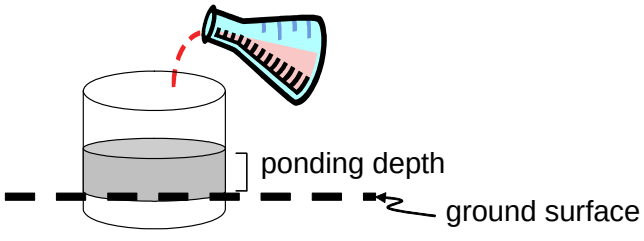
A. Infiltration test

1. Select a level site for your test. Remove loose debris (leaves, sticks) from an area the size of your infiltrometer (but do not pull up rooted plants; this will affect the pores in the soil).

2. Insert the ring infiltrometer several centimeters into the soil. Record this penetration depth. The ring should be inserted deeply enough and sealed adequately to the soil to preclude any leakage from the ring.

3. Fill out the top of the data sheet to record your group members and experimental set up.

4. To conduct the infiltration test, establish a standing pond of water within the ring that you maintain to within about 10% of this depth throughout the test. Once you have established this ponding depth, add water to maintain a constant ponding depth throughout your experiment. This should require frequent additions of water at the start of your experiment and less frequent additions as your test proceeds. Continue to make measurements of water additions for at least one hour, recording additions at least every 10 minutes, but more frequently if needed to maintain a constant ponding depth.



B. Soil extraction for bulk physical properties

1. Immediately adjacent to each of your infiltration tests, extract a bulk sample of the mineral soil using the soil auger. Retain only the center ring of your extracted sample. Be sure to record the dimensions (diameter, length) of the device used to extract your sample.

2. Place the sample into a plastic bag, labeled with your name(s) and indicate whether it is from the "forest" or "trail" site.

3. In the lab, weigh an empty aluminum pan to determine the tare weight, then place your sample in the pan and weigh again. Place the soil sample in the oven for overnight drying at 103^{o}C. When drying is complete, weigh the sample again to determine dry weight.

C. Data reduction, analysis and interpretation

1. Use the data reduction instructions following each data sheet to make calculations from your raw field data.

2. Enter your infiltration data for both sites into a spreadsheet with columns to record time, elapsed time, volume of water added, and depth of water infiltrated at each time step. Your entries should include at least one hour of observations.

3. Plot the data in your spreadsheet as an x,y scatterplot with elapsed time on the x axis and infiltration rate on the y axis (see for example figure 5.4 in your textbook).

4. Estimate a steady state infiltration capacity from your data plot for both sites by taking an average of measurements over a time interval during which infiltration rate shows little or no change.

5. Consider/discuss:
   - How do the steady state infiltration rates differ between the two sites you measured?
   - What factors influence the rate at which infiltration occurs; how do your measurements of bulk density relate to any of these factors?
   - What are the limitations associated with inferring infiltration rates across the landscape based on the measurements you have made?

Infiltration Test Data Sheet

Group member names: _____

Experiment date: _____ Location: _____

Experimental Set-up

Diameter of infiltrometer (d): _____

Site type: forest | trail

Infiltrometer length (cm) _____ Depth inserted into soil (cm) _____

Ponding depth (cm) _____

DATA:

time (hr:min:sec)	volume start (ml)	volume end (ml)	volume added ( )		time (hr:min:sec)	volume start (ml)	volume end (ml)	volume added ( )
0:00:00	--	--	--					
	1000							
			.					.

Adapted from Measuring Infiltration Lab by Beverley Wemple, UVM Geography Department.
Please do not distribute without permission.

Infiltration Test Data Sheet

Group member names: _____

Experiment date: _____ Location: _____

Experimental Set-up

Diameter of infiltrometer (d): _____

Site type: forest | trail

Infiltrometer length (cm) _____ Depth inserted into soil (cm) _____

Ponding depth (cm) _____

DATA:

time (hr:min:sec)	volume start (ml)	volume end (ml)	volume added ( )		time (hr:min:sec)	volume start (ml)	volume end (ml)	volume added ( )
0:00:00	--	--	--					
	1000							
			.					.

Data reduction:

To compute infiltration rates from your experiment, you will need to convert the volume of water to a water depth, then divide by the elapsed time. Follow the steps below to reduce your data and compute infiltration rates for each experiment. In each step, write the formula you use, then clearly show your calculations with units:

1. Calculate the surface area (A) of the infiltrometer from the diameter of the ring. (4 pts)

2. For one time step on one your data sheet, compute depth of water infiltrated (D) as the volume[1] of water (V) divided by the surface area (a) of the infiltrometer. Use an arrow on your data sheet to indicate the time step for which you are making this calculation. (4 pts)

3. For the time step used in #2 above, convert the elapsed time (t) in minutes and seconds to time in hours (this should be a fraction of an hour). (3 pts)

4. Compute infiltration rate (I) by dividing water depth (D) by elapsed time (t). Express your answer in cm/hr (4 pts)

[1] Note: Water volume for the experiment is measured in milliliters. 1 ml = 1 cm^3.

Bulk Density Data Sheet

Plot 1 (circle one): forest | trail

Auger ring diameter (cm) _____ Auger ring length (cm) _____

Sample tare weight (g): _____

Sample field weight (g): _____

Sample dry weight (g): _____

Notes on site conditions:

Plot 2 (circle one): forest | trail

Auger ring diameter (cm) _____ Auger ring length (cm) _____

Sample tare weight (g): _____

Sample field weight (g): _____

Sample dry weight (g): _____

Notes on site conditions:

Data reduction:

To compute bulk density and gravimetric moisture content, you will need to calculate the volume of soil extracted, then weigh it to get mass of the soil and mass of water lost with drying. Follow the steps below to reduce your data. For each step, write the formula you use and clearly show your calculations with units:

Forest site:

1. Calculate ring volume. (3 pts)

2. Calculate the bulk density of the soil sample. (3 pts)

3. Calculate the gravimetric moisture content of the soil sample. (3 pts)

Trail site:

1. Calculate ring volume. (2 pts)

2. Calculate the bulk density of the soil sample. (2 pts)

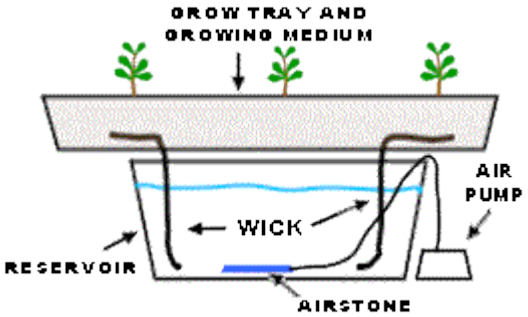
3. Calculate the gravimetric moisture content of the soil sample. (2 pts)

Source: http://www.simplyhydro.com/hydrou.htm

Basic Hydroponic Systems and How They Work

There are 6 basic types of hydroponic systems; Wick, Water Culture, Ebb and Flow (Flood & Drain), Drip (recovery or non-recovery), N.F.T. (Nutrient Film Technique) and Aeroponic. There are hundreds of variations on these basic types of systems, but all hydroponic methods are a variation (or combination) of these six. Scroll down this page (or click on the system names) to see drawings and a description of each type of hydroponic system.

WICK SYSTEM

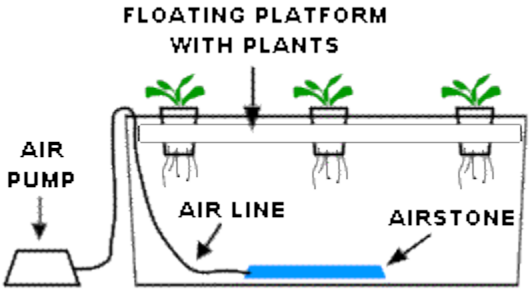


The Wick system is by far the simplest type of hydroponic system. This is a passive system, which means there are no moving parts. The nutrient solution is drawn into the growing medium from the reservoir with a wick. Free plans for a simple wick system are available (click here for plans).

This system can use a variety of growing medium. Perlite, Vermiculite, Pro-Mix and Coconut Fiber are among the most popular.

The biggest drawback of this system is that plants that are large or use large amounts of water may use up the nutrient solution faster than the wick(s) can supply it.

WATER CULTURE



The water culture system is the simplest of all active hydroponic systems. The platform that holds the plants is usually made of Styrofoam and floats directly on the nutrient solution. An air pump supplies air to the air stone that bubbles the nutrient solution and supplies oxygen to the roots of the plants.

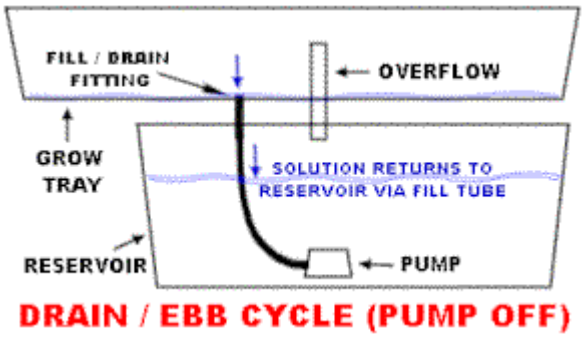
Water culture is the system of choice for growing leaf lettuce, which are fast growing water loving plants, making them an ideal choice for this type of hydroponic system. Very few plants other than lettuce will do well in this type of system.

This type of hydroponic system is great for the classroom and is popular with teachers. A very inexpensive system can be made out of an old aquarium or other water tight container. Sample plans are available here - http://www.simplyhydro.com/free2.htm.

The biggest drawback of this kind of system is that it doesn't work well with large plants or with long-term plants.

EBB & FLOW - (FLOOD AND DRAIN)

The Ebb and Flow system works by temporarily flooding the grow tray with nutrient solution and then draining the solution back into the reservoir. This action is normally done with a submerged pump that is connected to a timer.

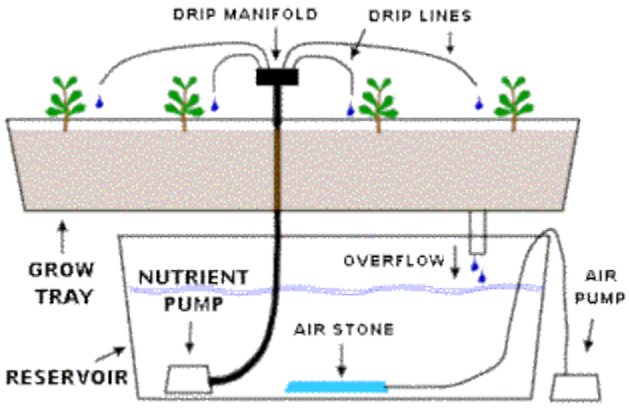


Source: http://www.simplyhydro.com/hydrou.htm

When the timer turns the pump on nutrient solution is pumped into the grow tray. When the timer shuts the pump off the nutrient solution flows back into the reservoir. The Timer is set to come on several times a day, depending on the size and type of plants, temperature and humidity and the type of growing medium used.

The Ebb & Flow is a versatile system that can be used with a variety of growing mediums. The entire grow tray can be filled with Grow Rocks, gravel or granular Rockwool. Many people like to use individual pots filled with growing medium, this makes it easier to move plants around or even move them in or out of the system. The main disadvantage of this type of system is that with some types of growing medium (Gravel, Growrocks, Perlite), there is a vulnerability to power outages as well as pump and timer failures. The roots can dry out quickly when the watering cycles are interrupted. This problem can be relieved somewhat by using growing media that retains more water (Rockwool, Vermiculite, coconut fiber or a good soilless mix like Pro-mix or Faffard's).

DRIP SYSTEMS RECOVERY / NON-RECOVERY



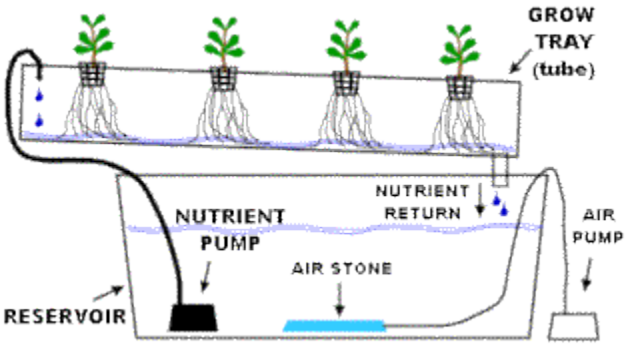
Drip systems are probably the most widely used type of hydroponic system in the world. Operation is simple; a timer controls a submersed pump. The timer turns the pump on and nutrient solution is dripped onto the base of each plant by a small drip line. In a Recovery Drip System the excess nutrient solution that runs off is collected back in the reservoir for re-use. The Non-Recovery System does not collect the run off.

A recovery system uses nutrient solution a bit more efficiently, as excess solution is reused, this also allows for the use of a more inexpensive timer because a recovery system doesn't require precise control of the watering cycles. The non-recovery system needs to have a more precise timer so that watering cycles can be adjusted to insure that the plants get enough nutrient solution and the runoff is kept to a minimum.

The non-recovery system requires less maintenance due to the fact that the excess nutrient solution isn't recycled back into the reservoir, so the nutrient strength and pH of the reservoir will not vary. This means that you can fill the reservoir with pH adjusted nutrient solution and then forget it until you need to mix more. A recovery system can have large shifts in the pH and nutrient strength levels that require periodic checking and adjusting.

N.F.T. (Nutrient Film Technique)

This is the kind of hydroponic system most people think of when they think about hydroponics. N.F.T. systems have a constant flow of nutrient solution so no timer required for the submersible pump. The nutrient solution is pumped into the growing tray (usually a tube) and flows over the roots of the plants, and then drains back into the reservoir.

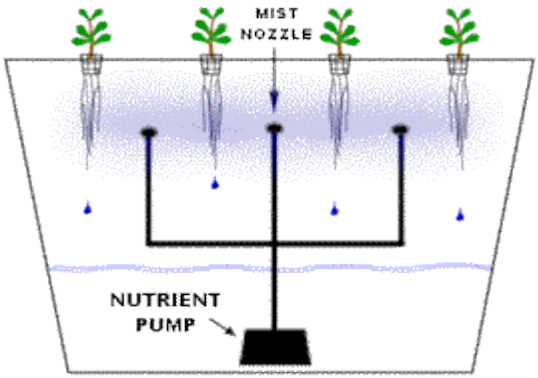


There is usually no growing medium used other than air, which saves the expense of replacing the growing medium after every crop. Normally the

plant is supported in a small plastic basket with the roots dangling into the nutrient solution.

N.F.T. systems are very susceptible to power outages and pump failures. The roots dry out very rapidly when the flow of nutrient solution is interrupted.

AEROPONIC



The aeroponic system is probably the most high-tech type of hydroponic gardening. Like the N.F.T. system above the growing medium is primarily air. The roots hang in the air and are misted with nutrient solution. The mistings are usually done every few minutes. Because the roots are exposed to the air like the N.F.T. system, the roots will dry out rapidly if the misting cycles are interrupted.

A timer controls the nutrient pump much like other types of hydroponic systems, except the aeroponic system needs a short cycle timer that runs the pump for a few seconds every couple of minutes.

Frequent Myths about Hydroponics

Myth: Hydroponics is a new technology

The Pharaohs of Egypt enjoyed fruits and vegetables grown hydroponically. One of the Seven Wonders of the Ancient World, The Hanging Gardens of Babylon, was believed to be a hydroponic garden. In India, plants are grown directly in coconut husk; hydro at the most grassroots level. If hydroponics is a "new" technology, it is a new technology in general use for thousands of years. Hydroponics is not new -- just different.

Myth: Hydroponics is artificial or unnatural

Plant growth is a real and natural happening. Plants require basic, natural things for normal growth. Hydroponics supplies the plant with what it needs, when it needs it. There is no genetic mutation that takes place inside the equipment nor are there any mysterious wonder chemicals introduced to the plants roots that trick them into thinking they're on steroids. With the production of more refined nutrients, it is now possible to grow completely organic produce with hydroponics.

Myth: Hydroponics is bad for the environment

This is false. As we are coming to realize that water is our most precious resource the first point worth noting is that hydroponics uses 70 to 90 percent ***LESS*** water than conventional gardening. The second greatest ecological benefit is that no fertilizer runoff escapes into our lakes, rivers and aquifers. These two items alone, water conservation and the non-pollution of lakes and streams, are major plus values.

Myth: Hydroponics is a space-science far too sophisticated and high-tech for the average person to understand or master

Hydroponics is growing without soil, and no bells or whistles are required to accomplish this. An inexpensive bucket or nursery pot, filled with a hydroponic growing medium and hand watered with a

hydroponic nutrient is hydroponics. A sheet of Styrofoam filled with net cups and floating on an aerated tank is hydroponics and as a point of fact, this system is very popular for elementary school science projects. The technological potential for automation and total environmental control is virtually limitless but in no way required to have a beautiful and abundant hydroponic garden. Basic hydroponics can be taught to the very young, the very elderly, and anyone open to learning a few new tricks.

Myth: Hydroponics must be used indoors

Hydroponics is as easy to use outdoors under the sun as it is indoors. One advantage to gardening indoors under grow lights is that you, not Mother Nature, control the seasons, making the growing season twelve months long. However, that is still true whether you grow in soil or hydroponically. Soil gardening can be done indoors and hydro can be done outdoors.

Myth: Hydroponics requires no pesticides

This is false. The need should be greatly reduced because a strong healthy plant is much less susceptible to attack than a weaker plant. Also, soil-born pests will be eliminated but even in an indoor environment, intruders still find their way in, catching a ride on your person or sneaking through tiny crevices. Monitor any garden carefully so you can catch problem insects when they first appear and your need for toxic products will be minimal.

Myth: Hydroponics produces huge super-plants

This myth has some foundation in truth but there is an important aspect to consider. Every seed, like all living things, already has a genetic code that will determine its general size, yield potential and flavor. Hydroponics can't turn a cherry tomato into a beefsteak tomato but it can turn it into the best cherry tomato it can be. Therefore, start with the best genetics possible.

Getting a plant to grow to its highest potential in common soil is difficult because of the hundreds of variables in the soil's make-up which influence the plant and its growth. It is the ability to control these variables that makes hydroponics superior to conventional gardening. In addition, factor that a plant in soil expends a great portion of energy working for its food in a way that hydro plants do not. The diva existence of a hydroponic plant allows it to send that extra energy into faster growth, dense vegetation, larger yields and more flavorful produce.

Myth: Hydroponics is used primarily for illegal purposes

Henry Ford once received a letter from a depression-era bank robber responsible for the deaths of several law enforcement officers, killed in their attempt to stop him as he fled the crime scene. In his letter, he thanked Mr. Ford for making his Model A Ford such a good getaway car.

Yes, hydroponics is popular with illegal growers. This popularity is founded on the same principles that make it popular with legal growers -- a bigger, better, higher quality crop.

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ESSAY REVIEW

Designs for greenhouse studies of interactions between plants

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Summary

1 Designs for greenhouse studies of interactions between plants are reviewed and recommendations for their use are provided.

2 Papers published over a 10-year period showed the replacement series design to be the most popular, especially in studying crop–weed interactions. Fifty per cent of the studies involved only two species, although studies testing the interaction between different genotypes of only a few species were also popular.

3 Limitations imposed by the choice of design, the variables measured, and the analysis used on the range of inferences that may be validly drawn from the experiment are frequently not well understood or appropriate for the questions that appear to be addressed. One example is the failure to distinguish the outcome of competition (the long-term outcome of interaction) and the effects of species on each other.

4 Studies in which only final yield is measured are severely limited as to the inferences which may be drawn. Effects due to interspecific interaction during the course of the experiment cannot then be separated from pre-existing differences, and interpretation may be biased towards species whose individuals were initially larger. In addition, measurements at several times are necessary to understand the changing dynamics of species interaction.

5 Simple pair-wise mixtures can assess the effect of treatment factors on the outcome of competition. Replacement series and related diallel designs generally produce results that may be size-biased even when initial interspecific differences are known. Additive designs (including target–neighbour designs), despite confounding density with species proportions, offer considerable scope for addressing mechanistic questions about interspecific interactions. Designs that allow response surface analysis can avoid many of the problems inherent in the other methods, but all need to be adjusted for initial interspecific differences. Designs for multiple species experiments are still largely untested, although several designs have been used. At the level of the individual plant, hexagonal fan designs permit study of the effects of varying the spatial pattern, and the densities and the relative proportions of interacting species, but suffer from lack of independence and lack of randomization.

Keywords: additive, competition, competitive hierarchy, diallel, experimental design, hexagonal fans, interspecific interaction, replacement series, response surface, size-bias, target–neighbour design

Journal of Ecology (1999) **87**, 1–16

Introduction

The importance of interactions between plants in determining the structure and dynamics of plant communities is widely recognized (e.g. Grime 1979; Aarssen 1983; Tilman 1988; Keddy 1989; Grace & Tilman 1990). However, demonstrating the effects of

these interactions in the field has often proved difficult (e.g. Strong *et al.* 1984; Connell 1990) so attention has tended to focus on studies of artificial communities growing in the greenhouse and in the field. While studies of plant interactions in natural and semi-natural communities have been the subject of several comprehensive reviews (Connell 1983; Schoener 1983; Underwood 1986; Aarssen & Epp 1990; Goldberg & Barton 1992; Goldberg & Scheiner 1993), greenhouse experiments have not been similarly reviewed, although the different approaches used have been described both in general terms (Harper 1977; Silvertown & Lovett Doust 1993) and for particular applications (Dekker *et al.* 1983; Radosevich 1987; Weidenhamer *et al.* 1989; Weidenhamer 1996). Statistical issues and questions have also been raised about the logical validity of some of the different designs as they are commonly used (e.g. Inouye & Schaffer 1981; Jolliffe *et al.* 1984; Connolly 1986, 1997; Rejmánek *et al.* 1989; Roush *et al.* 1989; Firbank & Watkinson 1990; Snaydon 1991).

Having surveyed appropriate journals published over a 10-year period for such experiments, we assessed the methodology (in particular the main experimental designs that have been used in studying plant–plant interactions under greenhouse conditions) and have made some recommendations for future practice.

Why study interactions of artificial communities in the greenhouse?

The complexity of natural plant communities imposes logistic and analytical constraints on studying plant interactions. For example, large numbers of species may be present, both environmental factors and species abundance show heterogeneity in time and space, and the size and age of the plants present will vary. By contrast, specially created artificial plant communities consisting of a few species, perhaps arranged in a particular pattern, with the plants of a specified age or ontogenetic stage, and with environmental conditions quite uniform and carefully controlled, can be used to examine inter- and intraspecific interactions more precisely. Further advantages of such controlled conditions are that the effects of other factors (e.g. soil fertility, pathogens and herbivory) can be more readily evaluated (Keddy 1989), and that such studies enable mechanistic interpretation rather than simple phenomenological observation (Tilman 1987; Stiling 1992).

The high degree of experimental control, repeatability, precision and amenability to rigorous statistical design make the use of artificial communities and greenhouse experiments appealing (de Wit 1960; Harper 1983; Hairston 1989), although others (e.g. Diamond 1986) have stressed the undoubted limitations. In particular, the lack of realism restricts the ability to apply the results of such experiments to complex natural communities: long-term greenhouse experiments with perennial plants can be especially unrealistic due to the inflexible restriction of rooting volume. However, such studies do allow the separation of different components of species interaction, such as effect and response (*sensu* Goldberg 1990), and determination of relative efficiency (Connolly *et al.* 1990). In addition, the mechanisms of interaction (e.g. through root and shoot capture of resources) are more amenable to study under controlled conditions. Despite the limitations, unless plant interactions can be demonstrated under greenhouse conditions they are unlikely to be of importance in natural communities.

Framework for the review

We are conscious that several aspects of the methodology used in the study of plant interactions have engendered heated debate. Replacement series (RS) or substitutive designs (de Wit 1960), in which one species gradually replaces another in a mixture at constant overall density, have been much used but no consensus has emerged since the breakdown in general acceptance of this design. In our opinion the limitations of other approaches have been glossed over and, in general, there has been an inadequate appreciation of the limited nature of inferences that can be drawn from several such techniques that have been widely used. We do not presume to offer a resolution of all issues in this review. Indeed, we do not believe that there is currently available in the literature a full context for the resolution of the difficulties posed by the study of plant–plant interaction, but we hope that, by adopting a critical review of some of the issues, we will help to clear a path towards such a resolution. While agreeing with Cousens (1996) that 'it is illogical to condemn a group of experimental treatments for all purposes simply because of the ways in which some experimenters choose to interpret the results', we consider that a critical analysis of methods is essential if such misuse is widespread and if the area appears beset by deep confusion.

Strictly speaking, an interaction between two plants is any association between plants in a mixture that affects the net reproductive rate (R_o) of the component species (Silvertown & Lovett Doust 1993). However, this definition may be too restrictive in practice as in many studies R_o is not measured and inferences are made on the basis of vegetative characters (Jolliffe *et al.* 1984; although see Benner & Bazzaz 1987; Law & Watkinson 1987). We therefore use the term in the broader sense of any effect one species has on another. There are many forms of interaction and many terms are used to specify particular facets of interaction (e.g. competitive ability, suppression, enhancement, intensity and importance of competition), and many analyses/indices have been proposed to provide a quantitative measure of them.

Rather than a detailed review of definitions and methods, we wish to provide a more narrowly focused critique of current experimental practice with a view to demonstrating some important limitations and providing some pointers as to how they may be avoided. We regard this as a necessary starting point in creating a framework for studies of plant–plant interactions within which the definitions of different forms of interaction and the methods used to measure them will be free of the difficulties that are outlined below.

Although there may not currently be general agreement in the literature on the following key concepts, we feel that they contribute substantially to the creation of a valid framework for studies on interspecific interaction. Some of these points cause fundamental difficulties for particular approaches, while others merely limit the range of inferences that may be drawn from certain studies.

Distinction between 'outcome of competition' and 'effects of species on each other'

We deal with two main aspects of interspecific interaction. 'Outcome of competition' refers to the relative long-term success of species, i.e. the end point for the community in terms of its composition and we are concerned with what indications short-term experiments can give about this. 'Effects of species on each other' refers to the impact of each species on the other (Goldberg & Werner 1983; Goldberg 1990) and may be an important part of the process that determines the end point, but is distinct from it. So while these two aspects of interaction may often be related, they are not equivalent, and their study may require different techniques. We believe that they are regularly confounded in current practice.

Since competitive exclusion rarely occurs in short-term experiments, the primary indicator available, however inadequate it may be, of long-term prospects is increased dominance of a species in a mixture, i.e. greater gain in terms of greater output per unit input. Although an estimate of this may be made from a single mixture, assessment of the effects of species on each other generally requires the inclusion of a range of mixtures and/or monocultures in the design.

Many experiments use methods and indices (e.g. relative crowding coefficients, coefficients of aggressivity, relative yield total) that purport to reflect the outcome of competition (Keddy & Shipley 1989) but actually address the questions of effects of species on each other or an amalgam of both. Furthermore, the indices and analytical methods used are generally susceptible to bias because they ignore initial differences between components, and therefore tend to favour larger individuals (Connolly 1986; Grace *et al.* 1993).

The role of time, initial conditions and plant size/life history

Analysis on the basis of final yield alone may be misleading, as although final yield represents a summation of the effects of plant interaction over the course of the experiment, it may also partly reflect initial differences. Initial size differences must be discounted to assess plant interactions adequately over the experimental period and make the comparison fair to both species. The final per unit size of a species will depend on both initial size and interspecific interactions (e.g. by an asymmetric effect such as increasing its shading impact more than pro rata to its size). These effects can be included as part of the explanation of subsequent performance (e.g. Connolly & Wayne 1996). This double role and use of initial size allows the experimenter to deal with situations where species ontogeny, other life-history traits, or direct experimental manipulation (e.g. of sowing date) lead to considerable differences in size between species at the commencement of the experiment. The sole use of final yield will also miss dynamic changes in species interaction (e.g. Connolly *et al.* 1990; Turkington & Jolliffe 1996) and is possibly the single most neglected and important issue in current practice.

The difficulty with density

Many experimental designs (e.g. including most RS designs or some additive designs) equate species on the basis of their numbers. However, simple equivalence on the basis of density can introduce size bias (Connolly 1986, 1997; Silvertown & Dale 1991; Grace *et al.* 1993) and thus distort an assessment of interspecific relations (e.g. Connolly 1986; Snaydon 1991). Snaydon (1991) gives the extreme example of the nonsense of equating densities of oak trees and daisy plants. Size differences may of course reflect life-history traits or natural conditions, and, where present, must therefore be allowed for, for example in the double way suggested in the section above.

Competition and single mixtures

Most assessments of interspecific interactions have used a single mixture (usually 50:50) in addition to the relevant monoculture(s). Data from additional mixtures or monocultures may be useful if they allow generalizations (since interaction may depend on the proportions and densities of the components) and will increase the precision of estimation of what is observable in the single mixture. However, a problem arises when such extra data contradict the findings obtained for a particular mixture (e.g. Benner & Bazzaz 1987; an example in Connolly 1997). In other words, because of the issues of size and density equivalence raised in the second and third sections above, a monoculture may not always be the appropriate

reference point for assessing the interactions in a particular mixture.

Limitations on inferences (logical limitations vs. misuse)

There are very few useless experiments (Cousens 1996). However, the inferences that can be validly drawn from a particular experiment depend on the design used, the measurements taken and the analysis of the data. If these logical limitations to inference are not fully appreciated, e.g. the second and third sections above, then biased assessments will result. We distinguish these logical limitations, which lead to pushing the inferences beyond what the design and measurements would support, from inappropriate interpretation resulting from faults with the design *per se* (i.e. misuse) (Cousens 1996).

Predictive power

Most studies of interspecific interactions are short-term, frequently lasting less than a year, often measuring only vegetative growth rather than reproductive success, and based on one phase of the life-history of species, whilst largely ignoring the rest. We must not expect too much predictive power from such experiments, unless we are convinced that the phase being tested is critically important (e.g. vegetative biomass can be used as a measure of fitness in many annual plants; Goldberg & Fleetwood 1987). They will usually supply no more than indicators to the answers required by ecologists, although they may be of more direct use to the interests of agronomists (e.g. Shrefler *et al.* 1994).

Our discussion develops analyses of the relationships between (i) questions that appear to be asked in studies of interspecific interaction, (ii) variables that are measured, and (iii) the designs used, with a view to identifying the range and limits of questions that can be validly addressed using particular combinations of design and variables measured (J. Connolly, P. Wayne & F. A. Bazzaz, unpublished data). J. Connolly *et al.* concluded that the omission of initial information severely limits the inferences that can be drawn using several of the most common designs. In the case of RS, even with the provision of this initial information the range of inference is still quite limited, and in other designs the comparison of species may remain problematic. J. Connolly *et al.* also draw attention to the distinction between the outcome of competition and the effects of species on each other (see the first section above) as an issue that has led to confusion in interpretation of studies on species interaction.

Literature survey

We surveyed studies published during 1984–93, in 11 journals (*American Journal of Botany*, *American Midland Naturalist*, *American Naturalist*, *Canadian Journal of Botany*, *Ecology*, *Ecological Monographs*, *Oikos*, *Journal of Applied Ecology*, *Journal of Ecology*, *Journal of Vegetation Science* and *Weed Science*). Although the last paper in our survey was from 1993, we believe that the findings are still relevant at the time of the final revision to this paper. Ninety-nine studies contained a total of 107 experiments on plant–plant interactions conducted in a greenhouse (the citations and designs used in these studies can be found in *The Journal of Ecology's* archive on the World Wide Web (WWW): see recent issue for address. The studies selected were limited to those with interspecific mixtures, except that intraspecific mixtures were also included when different genotypes, varieties or maternal lines were investigated. For each study, the following information was noted: experimental design, number of species studied, and identity and number of experimental treatments.

Most of the 107 experiments (35%) used RS designs, with two other designs (additive and simple pair-wise, see later) accounting for most of the rest (26% and 22%, respectively, Table 1). Clearly, RS has been the most widely used design in agricultural studies or investigations of crop–weed interactions. *Weed Science* (28%) and *Journal of Ecology* (20%) were the most commonly used journals, with an additional 23% of the studies reported in *Journal of Applied Ecology* or *Ecology*.

Fifty per cent of the studies surveyed examined interactions in mixtures involving only two species (Table 2) and fewer studies were encountered as the number of species tested increased. Only two studies in our survey examined seven species (Rabinowitz *et al.* 1984; Goldberg & Landa 1991) and multi-species designs (by definition) regularly studied three or more species (six in two studies: Austin *et al.* 1985; Thórhallsdóttir 1990). One study used three species, each of 10 genotypes, in all possible two-genotype pairs according to a diallel design (Taylor & Aarssen 1990). Gaudet & Keddy (1988) used a modified additive design to measure the relative competitive ability of 44 herbaceous plant species, but this ambitious study was not included in our survey. Twenty general topics were addressed in the 107 experiments (Table 3). Crop–weed interaction was the most frequent (21 studies), with interactions between or among genotypes, and effects of soils and nutrients, also common.

Types of design

The experimental designs that have been used in studies of plant interactions have been classified in various ways (e.g. Harper 1977; Dekker *et al.* 1983; Radosevich 1987; Austin *et al.* 1988; Rejmánek *et al.* 1989; Firbank & Watkinson 1990; Snaydon 1991; Silvertown & Lovett Doust 1993). Despite the use of different terms, three main types of design are commonly recognized: simple pair-wise (SP), additive (AD) and

Table 1 Number of greenhouse experiments of plant interactions published in 99 studies in 11 leading journals from 1984 to 1993. AMN, *American Midland Naturalist*; AN, *American Naturalist*; AJB, *American Journal of Botany*; CJB, *Canadian Journal of Botany*; Ecol, *Ecology*; EM, *Ecological Monographs*; JAE, *Journal of Applied Ecology*; JE, *Journal of Ecology*; JVS, *Journal of Vegetation Science*; Wsci, *Weed Science*

Design	AMN	AN	AJB	CJB	Ecol	EM	JAE	JE	JVS	Oikos	Wsci	Total
Simple pair-wise		1	3	3	5	1		4		1	6	24
Additive		2	2	1	6		4	5		2	6	28
Replacement	3		1		3		6	7			17	37
Diallel		1	1	3								5
Fan								2				2
Multi-species mixtures				2	1			2	1	1	1	8
Other								2			1	3
Total	3	4	7	9	15	1	10	22	1	4	31	107*

*107 experiments are listed from 99 studies because some studies involved a combination of experiments and designs.

Table 2 Designs used and number of species tested in 99 greenhouse interference studies from 1984 to 1993

	Number of species							
Design	1	2	3	4	5	6	7	Total
Simple pair-wise	5	10	5	3		1		24
Additive	5	12	4	5	1		1	28
Replacement series		26	8	1		1	1	37
Diallel		2	3					5
Fan		2						2
Multi-species	1†		2	2	1	2		8
Other‡		2			1			3
Total	11	53	22	11	3	4	2	107*

*107 experiments are listed from 99 studies because some studies involved a combination of experiments and designs.
†A single test plant of *Ailanthus atlissima* was grown with germinating seedlings from old-field seed bank samples.
‡Not clearly classified as one of the designs listed above.

RS (also called substitutive designs). The differences are illustrated in Fig. 1. SP designs usually maintain a 1:1 ratio of the two competitors, whereas in the simplest case of AD experiments the density of one species is held constant while the density of the other species is varied. In RS, species are grown in varying proportions and compared to growth in monoculture, with the total density held constant across all mixtures/monocultures. A design for *n* species that consists of RS for all possible pair-wise combinations between the species is termed a mixture diallel design. Designs for response surfaces may consist of additive or substitutive designs at a range of densities, or may be constructed in other ways (e.g. Connolly 1987; Law & Watkinson 1987; Rejmánek *et al*. 1989; Roush *et al*. 1989; Snaydon 1991; Turkington & Jolliffe 1996). Less often used are spatially explicit designs (hexagonal fan designs) and those used to investigate multi-species interactions. Although not the focus of this review or our survey of the literature, our comments also have relevance for field experiments using these designs.

SP DESIGNS

In SP experiments (also called additive, equal proportions; Austin *et al*. 1988), mixtures consisting of a fixed, usually 1:1, ratio of the two species are maintained (Fig. 1a). SP designs have been used to examine the role of numerous factors in plant interactions, frequently using a range of treatments applied to a particular mixture of two species (see the WWW archive for examples). Additions of monocultures at appropriate densities can convert SP designs to AD (e.g. Gurevitch *et al*. 1990) or RS (e.g. Berendse *et al*. 1992) experiments. Some studies are difficult to classify as strictly SP, diallel or AD studies (e.g. Allen & Allen 1984, where the design is a partial diallel, with pair-wise comparisons of *Salsola kali* with two other species, but not between the other two species).

SP designs at a single relative frequency and density can be used, in a limited way, to address questions about the outcome of competition between two species. Measurements over time should be included to

Table 3 Topics addressed in 107 greenhouse studies published in 11 journals from 1984 to 1993. Several studies tested more than one factor

Topic	Number of studies
Crops and weeds	21
Genotypes	15
Soils and nutrients	15
Fungi, bacteria and diseases	9
Grazing	9
Moisture	9
Plant form and performance	8
Germination and seeds	7
Planting density	7
Spatial patterns	6
Abundance	5
Photosynthesis and light	5
Carbon dioxide	4
Modelling and data analysis	4
Roots	3
Herbicides	2
Leachates and allelopathy	2
Temperature	2
Breeding systems	1
Site of origin	1
Total	135

allow assessment of changes in relative abundance. However, SP designs do not allow assessment of the effects of species on each other, unless one or other species completely disappears. If final yield is the only parameter available then all that one can safely say is whether both species survived and which contributed most to final biomass. If an experiment includes pair-wise mixtures between more than two species, then comparisons of interspecific interactions for different mixtures may be problematic (J. Connolly *et al.*, unpublished data).

SP designs therefore provide a useful, if limited, tool for screening the effects of a treatment gradient on the outcome of competition; they are efficient in that no resources are allocated to monocultures, which may not provide useful information on the question addressed. In addition, they are amenable to fairly straightforward statistical treatment, and the difficulty raised by the probable correlation between responses in a mixture can often be avoided by combining the responses to give a single per-pot measure. Perhaps SP designs are used less frequently than they should be.

RS DESIGNS

In an RS, the planting density of the two constituent species may vary but the total density is held constant

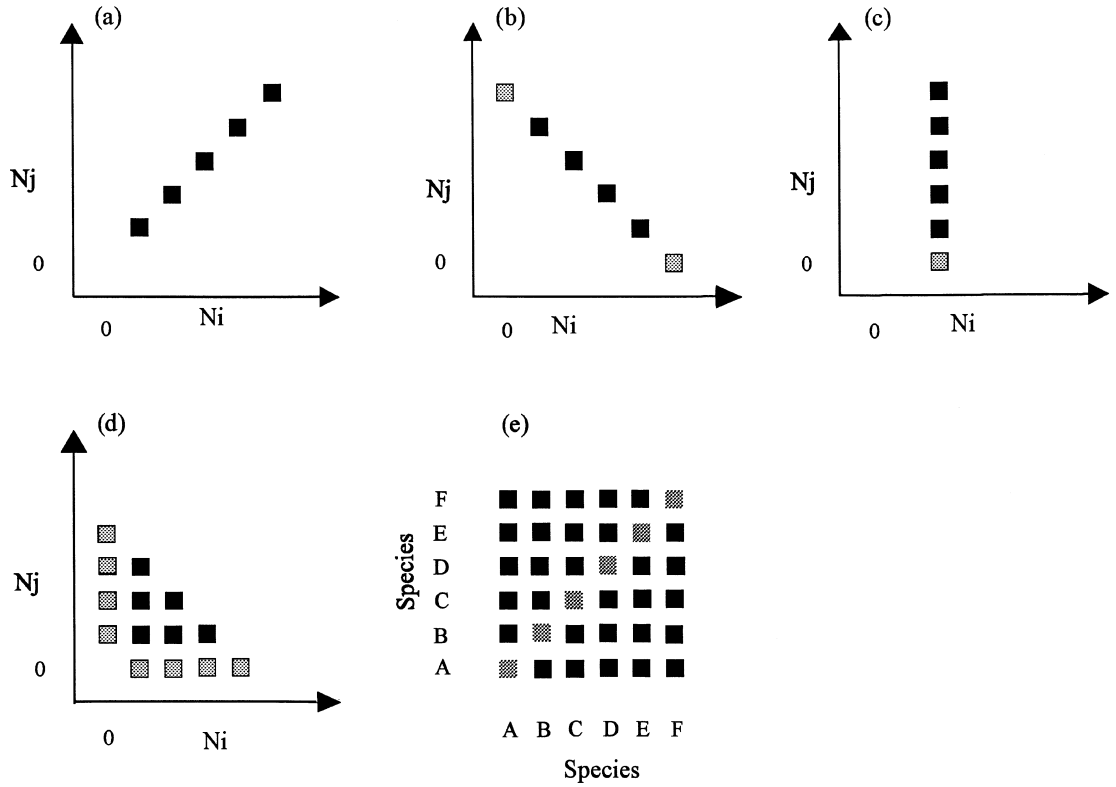

Fig. 1 Five designs of competition experiments plotted on joint abundance diagrams denoting the density (N) of species i and species j (designs a, b, c and d) and six component species in a diallel design (e) (after Rejmánek *et al.* 1989; Silvertown & Lovett Doust 1993). In (a–d) lightly shaded symbols represent monocultures. (a) Simple pair-wise (SP) design at multiple densities and without the monocultures included by some investigators; (b) replacement series (RS) at a single total density; (c) target–neighbour or partial additive form of an additive (AD) design with a constant density of component *i*; (d) additive series; (e) diallel design including redundant intraspecific mixtures (lightly shaded symbols).

(Fig. 1b). The effect of other factors (e.g. a soil nutrient) on interaction between the components is tested by using a replicate RS for each of several levels of the factor. Ratio and replacement diagrams (Harper 1977) offer graphical presentation of results. Of the several indices that have been proposed to present the results of RS experiments (Trenbath 1978; Connolly 1986), relative yield total (RYT; de Wit & van den Bergh 1965) is generally the most popular. The objective of some of these indices (e.g. relative crowding coefficients, de Wit 1960; competitive ratio, Willey & Rao 1980; coefficient of aggressivity, McGilchrist & Trenbath 1971) generally appears (although this is not often clearly stated) to be an attempt to assess the outcome of competition, whereas the RYT, a single value for the stand, relates to the joint capture and use of resources by the competing species (i.e. it describes a niche relationship). Although niche relationships contribute to understanding why a particular outcome occurs, they rarely predicate any particular outcome; thus the value of an index like RYT does not determine one way or another what impact niche separation will have on the outcome of competition.

Replacement designs have been widely used since they were introduced by de Wit (1960). Applications include aspects of inter- and intraspecific interactions between wild plants (e.g. Solbrig *et al.* 1988; Fone 1989), between wild plants (weeds) and crops (e.g. Ogg *et al.* 1993; Wall 1993), and between commercial cultivars of forage grasses (e.g. Frankow-Lindberg 1985). In the majority of studies, yield is the only character assessed, although other measures such as shoot/root ratios may perhaps help to illustrate the physiological basis of species' interactions (Bi & Turvey 1994).

We identified five problems with the RS design that seriously undermine its usefulness as an experimental tool (see also Cousens 1996). (i) It is generally used with final yields only, which can lead to size bias in interpretation if species differ in initial size (Connolly 1986, 1997; Grace *et al.* 1992; but disputed by Shipley & Keddy 1994). (ii) The validity of the RS method rests on the assumption that individuals of the competing species are exactly equivalent at the start of the experiment (Keddy 1989). If seedlings are of quite different sizes then it is both difficult to see how they can be regarded as equivalent (Connolly 1986; Snaydon 1991) and impossible to eliminate size bias (J. Connolly *et al.*, unpublished data). (iii) The outcome of competition is frequently confused with the effects of neighbours when interpreting results of RS. Including information from monocultures in the analysis can introduce bias in the assessment of species' effects both on each other in a mixture and on the long-term outcome (Connolly 1986; J. Connolly *et al.*, unpublished data). (iv) RS are carried out at a fixed, and often arbitrarily chosen, density (Inouye & Schaffer 1981; Taylor & Aarssen 1989; Snaydon 1991; Silvertown & Lovett Doust 1993) and results at that density may not generalize. Some of the density problem can be overcome by using replicate RS at different total densities derived from an additive series over a range of densities (Fig. 1d) (Firbank & Watkinson 1985; Cousens & O'Neill 1993). (v) Logistically, RS experiments necessitate tying up large numbers of experimental units (66% if only a singe mixture is used) in monocultures that may not contribute significantly to the analysis.

These problems lead to difficulty in correctly interpreting both RS diagrams and competition indices (Connolly 1986, 1988, 1997; Snaydon 1994). We are led to agree with the critics of this method (e.g. Inouye & Schaffer 1981; Jolliffe *et al.* 1984; Connolly 1986, 1988, 1997; Law & Watkinson 1987; Snaydon 1991, 1994): while RS may yield some useful information (Cousens 1996) it will be on a very limited range of questions. The tendency to misuse the method is so pervasive that its continued use should be discouraged.

AD AND TARGET–NEIGHBOUR DESIGNS

In the simplest form of AD designs (i.e. the partial additive) the density of the focal species is maintained across all mixtures and the density of the associate species is varied, usually with the goal of assessing the response of the focal species to increasing levels of the associate (Fig. 1c). More complex designs involve simultaneously varying the proportions of focal and associate species (i.e. addition series; Fig. 1d). This approach has useful applications, such as studying the impact of varying densities and distributions of weed populations on a crop sown at fixed density (Zimdahl 1980; Radosevich 1987; see the WWW archive). Additive designs have also been used to assess the role of various factors (e.g. relatedness, genotype, emergence time, initial plant size, maternal effects, herbivory) on a focal species' response to its associate in situations where comparing intra- vs. interspecific interactions and distinguishing effects of species' proportions from those of total density were less important objectives (see the WWW archive). They have been used for distinguishing allelopathic effects from resource exploitation due to density-dependent phytotoxic effects (Weidenhamer 1996; Weidenhamer *et al.* 1989).

A problem with this design is that the overall density and the proportions of focal and associate species can vary simultaneously and this confounding of variables makes the interpretation of results difficult (although not necessarily unrealistic compared with field situations) (Harper 1977; Silvertown & Lovett Doust 1993). Some of the problems of confounding the effects of species' proportion and density can be overcome by independently manipulating densities of both species and analysing performance of the focal

species via response surface methods (Firbank & Watkinson 1985; Law & Watkinson 1987; Fredshavn 1994).

Target–neighbour designs involve growing an individual of a 'target' species with varying abundances of 'neighbours', which could be either an associate species or itself. This is essentially an AD design in which the density of the focal or target species is reduced to a single individual or to a density low enough to preclude significant intraspecific interactions. This design has been used to address a variety of mechanistic questions about plant interactions (see the WWW archive for examples). Goldberg & Landa (1991) used it to determine which plant traits are responsible for differences in the effects and responses between species, and whether these two measures of interaction are related.

The per unit (per capita or per unit biomass) effect of neighbours on individuals of a target species is measured as the slope of a regression of target plant performance against the number (or biomass) of immediate neighbours. The target–neighbour design focuses on individual plant responses rather than the mean population response and estimates the importance of interspecific interactions relative to other factors in determining the fate and performance of individuals. While these measures on the target do give information at the individual plant level, they do not allow direct assessment of the outcome of competition for the target since the factors affecting the target may also affect the neighbours to the same or greater degree. For example, increasing the density of an associate may greatly reduce the performance of the target but it may also reduce the performance of the associate. Comparison of the impact on both species is essential in assessing the outcome of competition.

This approach claims numerous additional advantages. By measuring interference on a per unit basis it incorporates asymmetries in individual plant size at harvest among competing species. The relationships can be useful in interpreting features of interspecific interactions. Comparison of the slopes of the target performance–neighbour abundance regressions can be used as a quantitative measure of the effect (*sensu* Goldberg & Fleetwood 1987) of different neighbour species (Goldberg & Landa 1991). Statistical comparison of these slopes under different conditions may be made using ANCOVA (e.g. Hartnett *et al*. 1993). However, as in all ANCOVA, care must be taken in interpretation if the covariate is estimated after the commencement of the experiment as it may also contain effects of treatments that are discounted in the comparison of slopes. For example, the method compares the effects of two associate species on the target as if they had the same final yield and, if this is not the case, may lead to an unfair comparison. The competitive 'response' can be estimated from the slopes of regression coefficients when different target species are grown with the same neighbour species (Goldberg & Landa 1991; Hartnett *et al*. 1993). This general approach has been described in some detail by Goldberg & Werner (1983) for use in field-based studies. Discussion of some statistical considerations for these types of additive experiments is found in Goldberg & Scheiner (1993).

An advantage claimed for target–neighbour experiments is their economy in terms of both space and plants (Thijs *et al*. 1994; compare Hartnett *et al*. 1993 with Hetrick *et al*. 1994). However, caution is necessary in claiming greater efficiency for one design over another. The statistical criterion used to compare the efficiency of different designs should in each case be the experimental resource required to achieve a particular precision in the estimation of a particular parameter(s). However, considerations other than statistical efficiency may influence the selection of design and measurement: a design that is somewhat less efficient for one particular purpose may provide a far wider basis for inference and may thus be usable to address a wider range of questions.

A variation of the target–neighbour approach incorporates measurements of the distance, as well as biomass or numbers, of neighbours, and so allows the decreasing effects of 'non-nearest neighbours' to be incorporated.

In practice, AD and target–neighbour designs often consider only final yield (but see Gibson & Skeel 1996) and so suffer from ignoring the time–course of interactions and initial differences in species' size. As well as confounding species density and relative frequency, they sometimes equate species simply on the basis of density (e.g. in comparing regression coefficients for different neighbour species where density is the independent variable in the regression). Thus conclusions may well be affected by size bias in a manner similar to RS, leading to certain species being judged more competitive simply because they were initially larger. Even if all information on initial sizes is available, a partial additive or additive series will not allow the same range of questions to be addressed as a response surface approach would (e.g. Connolly & Wayne 1996).

Despite the biases that can occur with these methods, comparisons among a range of treatments applied to the same additive series and species will give a basis for ranking treatments relative to each other, even if the absolute level of the effects may be biased. However, comparisons of treatments across species potentially suffer from difficulties unless initial size differences are measured and accounted for.

RESPONSE SURFACE METHODS

An experimental design that includes a range of densities and relative frequencies of the species under study (not necessarily including any monocultures, e.g. Connolly & Wayne 1996; Ramseier *et al*. 1996)

may be used to generate response models for each species. Such a design allows the fitting of regression-style response models relating some measure of per capita performance for each species to the density of each species (e.g. Suehiro & Ogawa 1980; Spitters 1983; Connolly 1987; Law & Watkinson 1987), the initial biomass of each species (Connolly & Wayne 1996) or some other initial measure of biological potential, such as early leaf area index of each species (e.g. Kropf & Spitters 1991). The response models and their parameters are used to assess species interaction. These methods avoid some of the problems inherent in the analysis of replacement and additive designs, and in diallel analysis (e.g. Law & Watkinson 1987; Bullock *et al*. 1995; Connolly & Wayne 1996). As with additive designs, the inclusion of initial and intermediate measurements allows the study of species' interactions over time. Connolly *et al*. (1990) and Menchaca & Connolly (1990) report changes in species' interactions over time that would have been totally overlooked in an analysis of final yield only. Indeed, the conclusions drawn from a response surface analysis incorporating the time–course of plant–plant interactions can be qualitatively different from, and more effectively predict the outcome of competition than, those derived from an RS (Connolly *et al*. 1990; Grace *et al*. 1993). The inclusion, for example non-destructive leaf demographic measurements, can provide a tool for time series/growth dynamics to be made.

Several ways of designing experiments for response surface models have been described. These include establishing an RS at several total densities, called an addition series (e.g. Spitters 1983; Connolly 1987; Radosevich 1987; Rejmánek *et al*. 1989; Rodriguez 1997) or establishing additive series (Fig. 1d, which can be regarded as either an additive design or a number of RS at different densities), similar to the bivariate factorial defined by Snaydon (1991). Any set of mixtures that allows the fitting of bivariate response models will suffice. In the absence of a statistical assessment the choice of optimal method is a moot point and may vary with the question being addressed.

Despite their definite superiority to RS and AD designs and methods, the response surface methods may also suffer from similar size bias in the estimations of species' effects and responses and the outcome of competition, unless initial differences are allowed for and the appropriate response measurements are analysed (J. Connolly *et al*., unpublished data). An example that corrects for initial differences is given in Connolly & Wayne (1996). Furthermore, there are several statistical issues in the fitting of some of these models (and those for AD), e.g. there is often a decrease in variance with decreasing plant size (Connolly *et al*. 1990) that should be allowed for. In estimating hyperbolic yield–density relationships it is preferable to use weighted regression, non-linear methods or the generalized linear model approach (Nelder & Wedderburn 1972) available in many statistical packages.

DIALLEL DESIGNS

Diallel designs use 'all possible combinations of n species', i.e. '$n(n-1)/2$ separate RS of two species, each represented by two pure stands and one equiproportioned mixture' (Harper 1977, p. 268; Trenbath 1978; but see also Gleeson & McGilchrist 1980 for unequal proportion extensions). Interspecific interactions are assessed using RS methods (Gurevitch *et al*. 1990) or by analysing a matrix of species' performance using ANOVA and/or covariance analysis (Trenbath 1978). A matrix of 'competition coefficients' (*sensu* Firbank & Watkinson 1985) calculated as slopes of regressions in a series of pair-wise target–neighbour experiments can also be analysed by diallel methods. Data from diallel designs carried out at more than one density may be analysed by response surface methods (Connolly 1987).

Diallel designs are derived from genetic analysis (Durrant 1965) and have been used extensively in the greenhouse and field by plant breeders and agronomists to assess interspecific interactions between cereal varieties and among forage grasses (e.g. Norrington-Davies & Hutto 1972; Rousvoal & Gallais 1973). Applications to better understand natural systems include Aarssen's (1988) study of four pasture species, Taylor & Aarssen's (1990) study of the interactions among 10 genotypes of three perennial grasses, and Aplet & Laven's (1993) study of the competitive hierarchy of four Hawaiian shrubs. The debate on competitive hierarchies (see below) relies heavily on results from experiments using diallel designs.

The diallel design at a single density is subject to the same difficulties in interpretation as RS.

HEXAGONAL FAN DESIGNS

Most experiments on interspecific interaction focus on the mean population responses of species (e.g. species yield) under varying densities and species' proportions. However, an important feature of plants and other sessile organisms is that they do not sense or respond to overall population density or frequency, but only interact with their immediate neighbours (Harper 1977). This principle argues strongly for designs, such as the target–neighbour and fan designs, that focus on the interaction between a plant and its immediate neighbours. Mead (1979) lists five spatial factors that may affect interspecific interactions between two species and hence can be included as factors in design, namely the density and the intraspecific spatial arrangement of each species and the intimacy of their interspecific arrangement. There are many approaches to the study of intraspecific inter-

actions between individuals (Firbank & Watkinson 1987), and some of the statistical issues were reviewed by Mead (1979). Fan designs were the only ones found in our literature survey.

Hexagonal fan designs utilize a particular plant spacing pattern involving a honeycomb of overlapping hexagons such that each individual is surrounded by zero to six intraspecific neighbours and six to zero interspecific neighbours. This array of hexagons is arranged in a plant spacing gradient (fan design) with plants positioned in a particular pattern, such as a polar coordinate grid or a parallel row design (Nelder 1962; Bleasdale 1967). Thus fan designs vary density and frequency and select a particular form for intraspecific spatial arrangement and interspecific intimacy (see illustrations in the studies listed in the WWW archive).

Hexagonal fan experiments developed as a combination of the fan designs used in agronomic trials to examine the effects of plant density (Nelder 1962), and hexagonal planting designs were developed by Boffey & Veevers (1977) to study the effects of neighbour species' frequencies. Thus they have the advantages of incorporating variation in species' proportions and densities and the local spatial distribution of neighbours in assessing the response of individual plants to neighbours. In addition, they can be significantly more efficient in use of greenhouse space relative to other designs (Antonovics & Fowler 1985). Schmid & Harper (1985) used a fan design to show that interspecific interactions change in varying ways with changing density, sometimes with complete reversals of competitive outcomes between two species at different total densities. In addition, hexagonal fan experiments help in the assessment of optimal planting arrangements in mixed-cropping systems and facilitate the analysis of frequency and density-dependent selection in genotype mixtures (Antonovics & Fowler 1985).

The primary advantages of hexagonal fan designs are their focus on neighbourhood interactions, their efficiency in use of space and plants, and their ability to allow assessment of interspecific interactions across a range of densities or plant spacing patterns. However, there are statistical problems associated with the analyses of such designs (Mead 1979; Antonovics & Fowler 1985): they are unrandomized and so may be biased due to underlying trends in fans, the correlated responses in neighbouring plants may require a more complex analysis, and they may have limitations in situations in which second or third nearest neighbour effects and more diffuse interactions are significant. Often the analysis of these designs assumes that 'non-nearest neighbour' effects are insignificant. In addition to these statistical difficulties, size bias may arise if initial size differences are not discounted. Like all studies of individual rather than mean response, they require a greater input of time and labour. These designs can be extended to study multi-species interactions (see below).

DESIGNS TO ASSESS MULTI-SPECIES INTERACTIONS

Despite attempts to provide the greatest degree of realism to interaction experiments, greenhouse experiments involving interspecific interactions among mixtures of three or more species, i.e. diffuse or multi-species interactions (MacArthur 1972), have been infrequent. This is perhaps not surprising given the logistical and statistical problems inherent in the effective design and interpretation of just the multiple pair-wise interaction experiments of the diallel design (Mitchley 1987). The growth of multi-species mixtures under various treatments can be used simply to assess the outcome of competition (e.g. Grime *et al.* 1987), but we have identified five further main approaches to assessing multi-species interactions in the greenhouse.

(i) Fowler (1982) showed that, in a three-species RS design (de Wit 1960), predicted yield per plant was statistically related to the observed yield per plant. Interpretations agreed with results obtained from pair-wise RS experiments. This method is subject to the same criticisms as the RS with two species. (ii) The performance of each species in a multi-species mixture compared with its performance in monoculture is a form of multi-species AD (see the WWW archive and Ellenberg 1954; Mueller-Dombois & Sims 1966; Pickett & Bazzaz 1978; Austin 1982). (iii) Rejmánek *et al.* (1989) applied reciprocal yield regression models to a three-species complete additive experiment but the results did not support the interpretations drawn from previous two-species investigations of the species. (iv) Plants can be grown in hexagonal arrays (see above) in which a species has each of the different species under investigation as a neighbour, but never itself. Thórhallsdóttir (1990) and Turkington (1994) used this approach to investigate the role of interspecific interactions on the spatial dynamics of grasses. While elegant, problems with this design include those mentioned above for hexagonal designs and others discussed in Thórhallsdóttir (1990). (v) Ramseier *et al.* (1996) proposed a simplex design (Cornell 1990) for multi-species experiments in which all species appear in each of a number of mixtures (the minimum number of mixtures is the number of species + 1) but in different relative frequencies, each species in turn being the largest component of a sown mixture with the other species being equally represented, with an additional mixture having all species equally represented. Repeated at a number of densities and with initial sizes of species measured, this design allows a response surface analysis in which questions of outcome and effects of species on each other may be assessed. Additional design points may be added and the order of interaction terms that can be assessed in the model depends on the structure and number of design points. Advantages claimed for the particular simplex design used are that each mixture is an experimental com-

munity with all species represented, that the spread in community type allows the examination of interspecific interaction over a wide range of systems, that resource use is efficient in that there are no resources devoted to monocultures, and that it can be readily extended to larger numbers of species in a coherent manner without a major increase in experimental size. Disadvantages are the possible complexity of a full statistical treatment. The problems raised earlier with other designs must be borne in mind when using any of these multi-species approaches.

Some other issues

BACKGROUND SPECIES

Interspecific interactions are sometimes examined by establishing a spacing gradient grid of one species and overseeding the entire grid with a second species. This attempts to assess the effects of varying intensities of intraspecific interaction under the constant influence of a 'background' (Radosevich 1987), although the idea of a constant influence on all species may be illusory. Such an approach ignores the reciprocal nature of many interspecific interactions, such that the introduced individuals generally influence the background species as well as being influenced by it. Over time the background will tend to respond differentially to different species and so what started as a common influence may rapidly cease to be so. This will occur at a localized level in the vicinity of the introduced individuals. Further, an individual or a unit of initial biomass will tend to have less effect at high compared with low density. In addition, a given density of a background species will have a smaller per unit effect on a fixed density of large rather than small introduced individuals of other species, since the overall effective density with large introduced individuals is greater than with small introduced individuals and so like is not being compared with like.

COMPETITIVE HIERARCHIES

Results from using several types of design (i.e. AD, target–neighbour, RS and diallel designs) have been prominent in the search for competitive hierarchies in which a species will out-compete (in the sense of outcome of competition) all species ranked below it in the hierarchy and be out-competed by those above it (Keddy & Shipley 1989; Shipley 1993). The occurrence of reversals of rank order, indicating either a network of competitive performance (intransitivity) (Herben & Krahulec 1990; Silvertown & Dale 1991; Shipley 1993) or competitive combining ability, is controversial (Taylor & Aarssen 1990). However, these designs as usually analysed are prone to the misinterpretations and dangers of size bias (Silvertown & Dale 1991; Grace *et al.* 1993; Connolly 1997; J. Connolly *et al.*, unpublished data), with factors such as differences in seed size or initial seedling mass providing a mechanism for that bias (although see Shipley & Keddy 1994).

Discussion/recommendations/conclusions: choosing the appropriate design

Greenhouse studies of plant interactions offer a number of practical advantages over field-based experiments, such as better control of treatments and extrinsic factors, that persuade us that they will have continued utility. Ideally, greenhouse studies should be carried out in conjunction with a field-based programme, and prior knowledge of how interactions take place in the field (e.g. densities, size differences, phenology, asymmetric effects) is necessary before planning an experiment.

Consideration of the six points proposed as a framework for this review lead us to the conclusion that experiments on interspecific interaction demand clarity in respect of the particular facet(s) of interspecific interaction that is the focus of the experiment (unambiguous terminology with precise data-based estimation of measures of those specific aspects of interaction), appropriate experimental design, measurement of appropriate variables and a correct analysis. Our survey indicated an insufficient appreciation of how these factors limit our ability to explore particular questions. While there is not yet available, in our view, a coherent approach to the difficulties posed by the study of competition, a better appreciation of some of the strengths and limitations in these areas is essential.

Inappropriate and inadequate experimental design and procedure in many studies have probably compromised our understanding of plant interactions. For example, the conclusions drawn from many RS experiments, especially those conducted at a single total density and/or based only on final yield, are unlikely to provide many meaningful ecological insights. Experiments where inappropriately sized individuals are matched against each other are similarly compromised. Although not a design issue *per se*, the confounding of terminology by investigators (e.g. definitions of competition vs. interference, outcome vs. effects, intensity vs. importance; Weldon & Slauson 1986) makes interpretation of experiments difficult. As an example we cite the distinction between the 'outcome of competition', the ultimate success or failure of species, and 'species' effects on each other' (possibly part of the explanation of the observed outcome) as one which rarely appears to be explicit but which has a direct impact on the design, analysis and interpretation of experiments. Additionally, a clearer realization of the limitations of short-term experiments in providing anything but simple indicators in respect of the outcome of long-term competition is desirable. Although not the focus of our review, it is also likely that inappropriate or incomplete analysis

of experimental data has limited the interpretation even of well-designed experiments (e.g. Watkinson & Freckleton 1997).

The choice of design, the variables measured and the analysis determine what questions can and cannot be answered, and should reflect the primary questions of interest. The major deficiency in this respect appeared to be the lack of recognition that many questions of interest could not be addressed adequately without introducing time as a factor. At a minimum there is the need to separate the effects of initial differences from those of subsequent interactions, which cannot be adequately done where only final harvest yield is available. Analysis based on final harvest yield alone can lead to size-bias in interpreting the results from AD, RS and response surface designs. Even when appropriate data on initial conditions are available, it may not be possible to produce unbiased information on some questions of interest for RS and AD designs (e.g. questions as to the outcome of competition).

We need to be very clear about the role of initial size in an experiment. Experiments can only measure effects from the time of establishment of the experiment to the final harvest time. If size differences between species exist at the start then it seems reasonable that the initial differences should be discounted in measures of performance over the experimental period, otherwise the measures are likely to reflect initial differences in addition to effects that arise during the course of the experiment. This is not to say that species that are initially bigger do not do better competitively on some per unit basis: they may, but the assessment of that should not be confounded with effects that simply reflect different initial sizes *per se*. Thus, for example, final yield per individual of a species depends both on its initial size and on its average Relative Growth Rate (RGR) through the course of the experiment. Greater initial size may lead to greater RGR due to an increased ability to compete for light, and so the final yield per individual is accordingly enhanced for larger individuals. This additional component of final yield (due to different initial size difference) must not be confounded, as it routinely is, with the mere scaling effect of initial size when comparing individuals or species that differ in initial size.

The effects of initial size differences can be allowed for by a double strategy of (i) using an initial biological measure such as total biomass of each species (Connolly & Wayne 1996) or total leaf area index for each species (e.g. Kropf & Spitters 1991) rather than density in response surface equations, and (ii) by using a per unit initial size measure of species' performance (e.g. RGR in Connolly & Wayne 1996). These approaches attempt to avoid difficulties arising from ignoring initial size differences and the use of density to equate species. They also focus attention explicitly on the influence of initial conditions and on the limited nature of inferences from this type of interaction experiment. Conclusions are valid only for the time during which the experiment was running, since what happened previously is built into the initial conditions and what happens afterwards is speculative.

Experiments based on single mixtures have been undervalued; even without information on initial conditions they can provide a simple, efficient method of addressing questions as to the changing balance between species along gradients of various kinds. When appropriate initial information is available a more powerful interpretation is possible. Single mixture experiments highlight the distinction between the outcome of competition, which can be approached within a single mixture, and interspecific effects, on which they generally provide no information.

While AD experiments, particularly those with target–neighbour designs, may need to be treated with caution if only final yield is available, they may suffice for certain objectives, e.g. to examine yield loss in crop–weed systems. However, the mechanism of this yield loss cannot be adequately addressed without allowing for initial conditions and, perhaps, taking intermediate measurements. For these same reasons, AD are inadequate and potentially misleading for some of the more evolutionary orientated concerns of ecologists. Even when information is available for several time-points comparisons of species as competitors against a range of target species may be compromised. AD do allow comparison of the rank order of treatment effects on some interspecific interactions but the absolute estimation of many interactions is beyond their scope if only final harvest data are used.

Despite much criticism in literature preceding or during the early years of the 10-year period surveyed (Inouye & Schaffer 1981; Jolliffe *et al.* 1984; Connolly 1986, 1988; Law & Watkinson 1987), we were surprised to find that the RS was still the most popular design. The problems with substitutive designs lead us to concur with Law & Watkinson (1987), Keddy (1989), Connolly (1986, 1988) and Snaydon (1991, 1994) that they should not be used for studies of plant interactions, except in very limited circumstances where it is clear that species are comparable in size at the start of the experiment. Even when RS are run at plant densities that closely match the range of natural abundances observed in the field, the fundamental problem remains that the same density is used for both species in monoculture (or an a priori and arbitrarily chosen x:1 ratio). Even if initial size differences are measured, the method cannot in general be corrected to produce valid results (J. Connolly *et al.*, unpublished data). When several such potentially size-biased studies are used to address an issue such as the existence and strength of competitive hierarchies, the scope for misleading inferences is clear. In the large number of studies on crop–weed interactions, it is surprising that RS experiments have been so widely

used compared to additive experiments (Sackville Hamilton 1994). The latter seem highly appropriate for crop loss studies since they take the form of a constant focal species (crop) density and varying associate species (weed) density (e.g. Thompson *et al.* 1994).

Response surface designs are widely seen as a generalization of AD and RS designs and as a remedy for their deficiencies. Even there, however, if only final harvest data are available the range of inferences is limited and the competition coefficients (Law & Watkinson 1987) or substitution rates (Connolly 1987) may include effects of initial differences between species as well as reflecting species' effects on each other.

There are many statistical issues beyond the scope of this review that need to be addressed in competition experiments, e.g. correlated responses, optimal design, estimation of response models and indices, but the first priority must be to ensure that the design, measurements, analyses and indices used lead to valid inferences. To make them more efficient is a secondary concern. Simple procedures have their attraction but more complex designs are necessary to address some questions. Such experimentation may have the advantage of a much wider scope for inference across a broader range of conditions.

We have taken care in this paper to focus rather narrowly on what we perceive to be some major difficulties with experimental procedures, and have not ventured into the deeper waters of deciding between competing theories (e.g. those of Grime 1979 and Tilman 1987) or the details of definition of subtle aspects of interaction, such as the distinction between the importance and intensity of competition (Weldon & Slauson 1986). We believe that a fuller appreciation of the way in which the design/variables/analysis complex determines the range of valid inferences must precede attempts to use experiments to support such theoretical positions or make such distinctions. We are led to this position by considering the confusion in the current literature, exemplified by the way in which possible size bias in RS and AD methods (Keddy & Shipley 1989; Herben & Krahulec 1990; Silvertown & Dale 1991; Grace *et al.* 1993; Shipley & Keddy 1994; Connolly 1997) has clouded the debate on competitive hierarchies.

We feel that perceptions of controversy and methodological turmoil have inhibited work on interspecific interactions, which is why this paper has concentrated on methodological difficulties rather than general prescriptions. The state of agreement is still not so advanced that we can move beyond the partial prescriptions of the previous few paragraphs, but at least some of the pitfalls are signposted. Once the issues are clarified the potential of these experiments on plant–plant interactions to provide reliable information will be released.

More sophisticated designs that incorporate time and allow response surface analyses with various biotic and abiotic explanatory variables and, perhaps, deal with several species in multi-species mixtures at individual and stand level, are going to be the most informative. Inclusion of root and shoot variables in models of species' performance should help elucidate their joint role in interspecific interaction and determine which facets of species, their growth, architecture, ontogenetic stage, limiting factors, etc., are most important in interspecific relations.

Multiple species investigations can be carried out through multiple pair-wise experiments (Goldberg & Scheiner 1993) or using multi-species designs (e.g. Ramseier *et al.* 1996). While the latter can be very rich in interspecific information they also carry more analytical and interpretative complexity and it may be too early to determine in what circumstances each is to be preferred.

Of course, increasing the complexity, both temporally and spatially, of experimental designs increases the logistical problems of carrying out the experiment. Simple experimental designs are preferable when they can validly address the questions of interest without an unacceptable sacrifice of realism. The caveat is that the results of such an investigation should be followed up by more sophisticated work, ideally including field experiments (e.g. Gibson & Skeel 1996; Skeel & Gibson 1998), before conclusions regarding the performance of plants in natural settings can be made with confidence.

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United States
Environmental Protection
Agency

Wastewater Technology Fact Sheet

The Living Machine®

DESCRIPTION

The Living Machine® is an emerging wastewater treatment technology that utilizes a series of tanks, which support vegetation and a variety of other organisms. The Living Machine® was conceived by Dr. John Todd, President of the non-profit organization Ocean Arks International, and gets its name from the ecologically-based components that are incorporated within its treatment processes (microorganisms, protozoa, higher animals such as snails, and plants). The Living Machine® has sometimes been referred to as the "*Advanced Ecologically Engineered System*" or AEES. The Living Machine® is now designed and marketed by Living Machines, Inc. of Taos, New Mexico.

The Living Machine® is a second generation design. Dr. Todd developed the Living Machine™ design concept after working on a number of similar small pilot-scale facilities, now referred to as Solar Aquatics™ and marketed by Ecological Engineering Associates of Marion, Massachusetts.

The Living Machine® incorporates many of the same basic processes (e.g., sedimentation, filtration, clarification, adsorption, nitrification and denitrification, volatilization, and anaerobic and aerobic decomposition) that are used in conventional biological treatment systems. What makes the Living Machine® different from other systems is its use of plants and animals in its treatment process, and its unique aesthetic appearance. While these systems are aesthetic appealing, the extent to which the plants and animals contribute to the treatment process in current Living Machine® designs is still being verified (U.S. EPA, 2001). In temperate climates, the process is typically housed within a large greenhouse, which protects the process from colder temperatures.

Source: U.S. EPA., 2001.

FIGURE 1 THE OPEN AEROBIC TANKS OF THE LIVING MACHINE® IN SOUTH BURLINGTON, VT

Living Machines, Inc. describes the Living Machine® as being a wastewater treatment system that:

- Is capable of achieving tertiary treatment;
- Costs less to operate than conventional systems when used to achieve a tertiary level of treatment; and
- Doesn't typically require chemicals that are harmful to the environment" as a part of its treatment process (Living Machines, Inc., 2001).

Several federally-funded Living Machine® demonstration systems have been constructed, the largest of which handled design flows of up to 80,000 gpd. As configured for these demonstrations, these systems treated municipal wastewaters at various strengths, and reliably produced effluents with five-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), and Total Nitrogen $\leq$ 10 mg/L, Nitrate $\leq$ 5 mg/L, and Ammonia $\leq$ 1 mg/L (U.S. EPA, 2001 and see Table 1). With regard to phosphorus removal, the Living Machine® process is capable of about 50 percent removal with influents within the 5-11 mg/L range (U.S. EPA, 2001). In addition to

the demonstration projects, the Living Machine® technology is being used by a variety of municipal and industrial clients, where similar performance has been reported.

Treatment Process

A typical Living Machine® comprises six principle treatment components, after influent screening. In process order (see Figure 1), these are (1) an anaerobic reactor, (2) an anoxic tank, (3) a closed aerobic reactor, (4) aerobic reactors, (5) a clarifier, and (6) "ecological fluidized beds" (EFBs). While the open aerobic reactors and EFBs are found in almost all Living Machines®, the other components are not always utilized in the treatment process. The specific components used are selected by the designers depending upon the characteristics of the wastewater to be treated and the treatment objectives. Sometimes additional process components may be added if considered necessary by the designers. For example, the demonstration system in Frederick, Maryland utilized a "Final Clarifier" and a high-rate subsurface flow (SF) wetland as the last two components of its treatment train.

Anaerobic Reactor (Step 1)

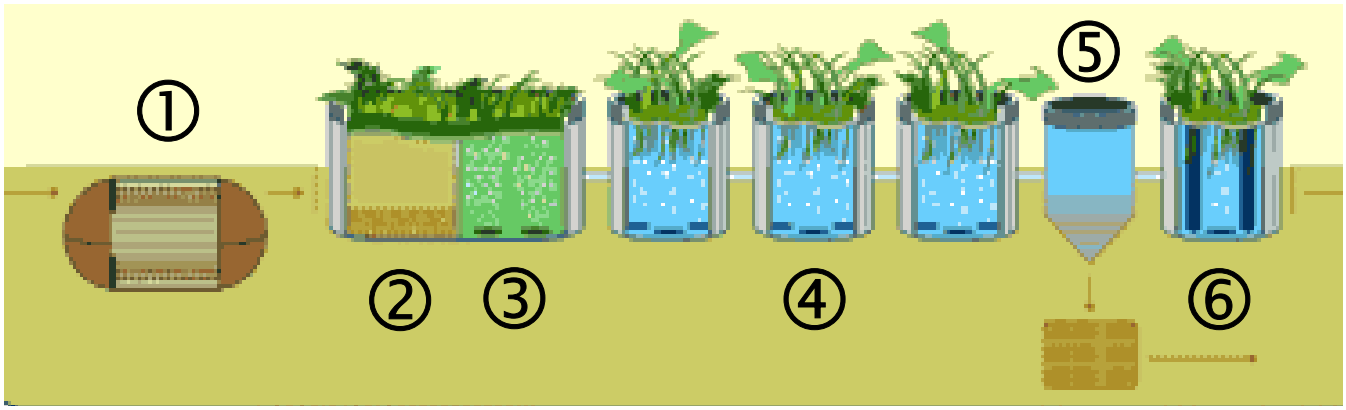
When it is employed, the anaerobic reactor serves as the initial step of the process. The reactor is similar in appearance and operation to a septic tank, and it is usually covered and buried below grade. The main purpose of the anaerobic reactor is to reduce the concentrations of BOD_5 and solids in the wastewater prior to treatment by the other components of the process. When necessary, gases are passed through an activated carbon filter to control odor.

Raw influent enters the reactor, which acts as a primary sedimentation basin. Some of the anaerobic reactors used have an initial sludge blanket zone, followed by a second zone for clarification. Additionally, strips of plastic mesh netting are sometimes used in the clarification zone to assist with the trapping and settling of solids, and to provide surface area for the colonization of anaerobic bacteria, which help to digest the solids. Sludge is typically removed periodically via perforated pipes on the bottom of the reactor, and wasted to a reed bed or other biosolids treatment processes. Gasses produced are passed through an activated carbon filter or biofilter for odor control.

Anoxic Reactor (Step 2)

The anoxic reactor is mixed and has controlled aeration to prevent anaerobic conditions, and to encourage floc-forming and denitrifying microorganisms. The primary purpose of the anoxic reactor is to promote growth of floc-forming microorganisms, which will remove a significant portion of the incoming BOD_5.

Mixing is accomplished through aeration by a coarse bubble diffuser. These diffusers are typically operated so that dissolved oxygen is maintained



Source: Living Machines Inc., 2001.

FIGURE 1 THE COMPONENTS OF THE LIVING MACHINE®: (1) ANAEROBIC REACTOR, (2) ANOXIC REACTOR, (3) CLOSED AEROBIC REACTOR, (4) OPEN AEROBIC REACTORS, (5) CLARIFIER, AND (6) "ECOLOGICAL FLUID BED"

below 0.4 mg/L. The space over the reactor is vented through an odor control device, which is usually a planted biofilter. Additionally, an attached growth medium can be placed in the compartment to facilitate growth of bacteria and microorganisms.

Settled biosolids from the clarifier (Step 5), and nitrified process water from the final open aerobic reactor (Step 4) are recycled back into this reactor. The purpose of these recycles is to provide sufficient carbon sources to the anoxic reactor to support denitrification without using supplemental chemicals, such as methanol.

Closed Aerobic Reactor (Step 3)

The purpose of the closed aerobic reactor is to reduce the dissolved wastewater BOD_5 to low levels, to remove further odorous gases, and to stimulate nitrification.

Aeration and mixing in this reactor are provided by fine bubble diffusers. Odor control is again achieved by using a planted biofilter. This biofilter typically sits directly over the reactor and is planted with vegetation intended to control moisture levels in the filter material.

Open Aerobic Reactors (Step 4)

Next in the process train are the open aerobic reactors, or aerated tanks. They are similar to the closed aerobic reactor in design and mechanics (i.e., aeration is provided by fine bubble diffusers); however, instead of being covered with a biofilter, the surfaces of these reactors are covered with vegetation supported by racks. These plants serve to provide surface area for microbial growth, perform nutrient uptake, and can serve as a habitat for beneficial insects and microorganisms. To what extent the plants enhance the performance treatment process in the Living Machine® is still being verified (U.S. EPA, 2001). However, with the variety of vegetation present in these reactors, these units (along with the Ecological Fluidized Beds - Step 6) set the Living Machine® apart from other treatment systems in terms of their unique appearance and aesthetic appeal.

The aerobic reactors are designed to reduce BOD_5 to better than secondary levels and to complete the process of nitrification. The size and number of these reactors used in a Living Machine® design are determined by influent characteristics, effluent requirements, flow conditions, and the design water and air temperatures.

Clarifier (Step 5)

The clarifier is basically a settling tank that allows remaining solids to separate from the treated wastewater. The settled solids are pumped back to the closed aerobic reactor (Step 3), or they are transferred to a holding tank, and then removed for disposal. The surface of the clarifier is often covered with duckweed, which prevents algae from growing in the reactor.

Ecological Fluidized Beds (Step 6)

The final step in the typical Living Machine® process are the "ecological fluidized beds" (EFBs). These are polishing filters that perform final treatment of the wastewater, and one to three are used in series to reduce BOD_5, TSS and nutrients meet final effluent requirements.

An EFB consists of both an inner and outer tank. The inner tank contains an attached growth medium, such as crushed rock, lava rock, or shaped plastic pieces. The wastewater flows into the EFB in the annular space between the inner and outer tanks and is raised by air lift pipes to the top of the inner ring that contains the media. The bottom of the inner tank is not sealed, so the wastewater percolates through the gravel media and returns to the outer annular space, from where it is again moved back to the top of the gravel bed. The air lifts also serve to aerate the water and maintain aerobic conditions.

The unit serves as a fixed bed, downflow, granular media filter and separates particulate matter from the water. Additionally, the microorganisms that occupy the granular media surfaces provide any final nitrification reactions.

As sludge collects on the EFB, it reduces its ability to filter. This would eventually clog the bed completely. Therefore, additional aeration diffusers

beneath the gravel bed are periodically turned on to create an upflow airlift, reversing the flow direction. This aeration is intended to "fluidize" the bed and release the trapped sludge (hence the name of this unit). This sludge is washed over and accumulated at the bottom of the outer annular space where it can be collected manually, and wasted along with the biosolids from the anaerobic reactor. Consequently, the name "ecological fluidized bed" is somewhat misleading for this unit since, in its treatment mode, it acts like a typical, conventional, downflow coarse media contact filter unit. Only during backwash cleaning does the bed become partially fluidized.

After this last step, the wastewater should be suitable for discharge to surface waters or a subsurface disposal system, or reused for landscape irrigation, toilet flushing, vehicle washing, etc. (Living Machines, Inc., 2001).

APPLICABILITY

The Living Machine® is well suited for treating both municipal and some industrial wastewaters. As with most treatment systems using plants, it can require a larger footprint than more conventional systems, and its requirement for a greenhouse in more temperate climates can impact costs. However, its unique and aesthetically pleasing appearance make it an ideal system in areas that oppose traditional treatment operations based on aesthetics (i.e., smell and appearance). The designers also stress the educational benefits of the Living Machine® (http://www.livingmachines.com/htm/planet2.htm) in raising awareness of wastewater treatment methods and benefits.

ADVANTAGES/DISADVANTAGES

Advantages

- Capable of treating wastewaters to BOD_5, TSS, and Total Nitrogen $\leq$ 10 mg/L, Nitrate $\leq$ 5 mg/L, and Ammonia $\leq$ 1 mg/L.

- Offers a unique, aesthetically pleasing environment for treating and recycling wastewater. This may be helpful when attempting to locate the treatment system in areas where the public opposes traditional wastewater treatment operations for aesthetic reasons.

Disadvantages

- The Living Machine® has only been shown to remove about 50 percent of influent phosphorous (with influents in the range of 5-11 mg/L). The removed phosphorus appears to be primarily associated with the incoming solids.

- The process requires a greenhouse for reliable operation in the cold weather of more temperate climates, adding to system costs.

DESIGN CRITERIA

Every Living Machine® system is designed by Living Machines, Inc. based upon the expected wastewater volume and content, as well as the treatment requirements and local climate. Once these factors are known, the designers then determine whether a greenhouse is necessary, what types of reactors are needed, how many of each type of reactor are required, and what capacity is required to achieve the suitable residence times.

PERFORMANCE

The Living Machine® has reliably achieved treatment goals of BOD_5, TSS, and Total Nitrogen $\leq$ 10 mg/L, Nitrate $\leq$ 5 mg/L, and Ammonia $\leq$ 1 mg/L. Table 1 shows the results of independent evaluations of two demonstration systems. The Living Machine® demonstration project in Frederick, Maryland was designed to treat 40,000 gpd of screened and degritted wastewater. It employed a single anaerobic reactor for primary solids digestion, then three parallel treatment trains, each comprised of two open aerobic reactors, a clarifier, three "ecological fluidized beds," a final clarifier, and a small, high-rate subsurface flow wetland. The demonstration project located in South Burlington, Vermont was designed to treat 80,000 gpd of screened and degritted wastewater,

TABLE 1 PERFORMANCE OF THE FREDERICK AND BURLINGTON LIVING MACHINES®

Parameter	FREDERICK				BURLINGTON			Effluent Goal
	Influent mg/L	GH Influent mg/L[a]	Effluent mg/L	% Removal	Influent mg/L	Effluent mg/L	% Removal	
BOD_5	230	156	4	97	227	5.9	97	<10
COD	944	378	21	94	556	35.9	94	--
TSS	381	70	2	97	213	5.3	98	<10
NH_3	-	22	1.2	94	16.3	0.4	98	<1
NO_3	-	20.8	10	52	15.9[b]	4.9	69	<5
TN (total nitrogen)	-	44	11	75	29.3	5.6	81	<10
TP (total phos-phorus)	11	7.7	6	45	6.0	2.0	67	<3

a Effluent from the anaerobic reactor at Frederick into the reactors contained within the greenhouse.
b Assumes that all removed ammonia is converted to nitrate.

Source: U.S. EPA, 2001.

and employed five open aerobic reactors (though one of these was later converted to an anoxic reactor), a clarifier, and three "ecological fluidized beds."

In these instances, the Living Machine® was capable of BOD_5 and TSS removal in excess of 95 percent. While the Frederick system did not consistently achieve its goal of < 5 mg/L for Nitrate, the Burlington Living Machine® did. The Living Machine® reliably demonstrated about 50 percent removal of Total Phosphorous (TP), although the Burlington system had a low influent TP concentration (U.S. EPA, 2001).

While the Frederick Living Machine® achieved quite good coliform removal (< 200 MPN/100mL), the Burlington system's effluent was above 1,000 MPN/100mL. Consequently, disinfection may be required as an additional step depending upon system configuration and effluent requirements.

OPERATION AND MAINTENANCE

Routine Activities

The routine operation and maintenance (O&M) requirements for Living Machines® are similar to the requirements for a conventional wastewater treatment plant, with a few additional requirements. These additional requirements include cleaning the inlet/outlet structure; cleaning the screen and tank; removing and disposing sludge; and maintaining and repairing machinery. Other requirements are vegetation management, including routine harvesting to promote plant growth, and removal of accumulated plant litter. Additionally, it may be necessary to manage fish and snail populations, and control mosquitoes and flies (if applicable).

Residuals Management

The Living Machine® produces residuals comparable in quantity to conventional treatment systems. However, some of these residuals are biosolids, while others are in the form of plant

TABLE 2 PRESENT WORTH COMPARISON OF "LIVING MACHINES®" AND CONVENTIONAL SYSTEMS

Process	40,000 gpd	80,000 gpd	1 million gpd
"Living Machine" with greenhouse	$1,077,777[1]	$1,710,280[1]	$10,457,542[2]
"Living Machine" without greenhouse	$985,391	$1,570,246	$9,232,257
Conventional System	$1,207,036[1]	$1,903,751[1]	$8,579,978[2]

(1) Cost difference is less than 20 percent
(2) Cost difference is greater than 20 percent

Source: U.S. EPA, 2001.

material. Analyses at the Frederick demonstration system showed that plant residuals could be composted and used for many agricultural or horticultural purposes. The biosolids would likely require stabilization and treatment to reduce pathogens and indicator organisms before they would meet Part 503 limits for sewage sludge (U.S. EPA, 2001).

COSTS

Since the Living Machine® is designed, marketed and trademarked by Living Machines, Inc., precise cost data are proprietary. However, a cost comparison with "conventional" treatment systems was performed as a part of an independent U.S. EPA evaluation of the Living Machines® (U.S. EPA, 2001). Table 2 summarizes the results of this cost comparison.

This analysis concluded that Living Machines® are typically cost competitive with more conventional wastewater treatment systems at flow volumes up to 1,000,000 gpd, if they are located in a warm climate where a greenhouse is not necessary. However, if the climate cannot support the plants year-round and a greenhouse must be constructed, construction costs will increase. Addition of a greenhouse structure makes the Living Machine® cost competitive with more conventional systems up to flow rates of around 600,000 gpd.

REFERENCES

Other Related Fact Sheets

Other EPA Fact Sheets can be found at the following web address:
http://www.epa.gov/owm/mtb/mtbfact.htm

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ADDITIONAL INFORMATION

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Dynamic control of supplemental lighting intensity in a greenhouse environment

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The global increase in energy prices, the urgent need to reduce CO_2 emissions to the atmosphere and the high energy usage are currently the major threats to the greenhouse industry. Optimised control of the lighting quality, quantity and periodicity can contribute to improvements in the productivity and energy efficiency of greenhouses. In this paper, the effects of dynamic control of supplemental lighting intensity on electricity consumption and fresh weight accumulation of lettuce plants are investigated. The use of the dynamic lighting control resulted in a 20% reduction in the electricity consumption in comparison to a similar lighting system operated under a discontinuous on–off regime. However, there was no statistically significant difference between both regimes in terms of plants' average fresh weight accumulated per electrical energy unit consumed.

1. Introduction

In northern latitudes, low availability of daylight and harsh weather conditions, especially during the winter season, are not favourable for year-round cultivation of vegetable and ornamental crops in the open field. Year-round plant cultivation is feasible in closed greenhouses where growth factors can be controlled independently of weather conditions or season of the year. It is known that the use of supplemental lighting in greenhouse cultivation has significantly contributed to improvements in the productivity of certain crops.[1–4] However, the use of artificial light to supplement or replace the lack of daylight also causes a significant increase in the total energy use of greenhouses. Due to the continuous increase in energy prices and the global need to reduce CO_2 emissions, improvements in energy usage are urgently needed. These necessary improvements in the energy efficiency of greenhouses are most preferably achieved by reducing the energy inputs (e.g. electricity and heating) without hindering crop productivity. It is also known that appropriate environmental control has a large potential to improve the energy efficiency of greenhouses.[5] Therefore, optimisation of supplemental artificial lighting can also contribute to address the current challenges that the greenhouse industry is facing (i.e. energy, environment and market).[6]

The growth and development of plants are influenced among other factors, by the quality (i.e. spectrum), quantity (i.e. intensity) and periodicity (i.e. time duration) of light exposure. The control of these parameters using artificial lighting is nowadays easier than in the past. The combination of light emitting diode (LED) lighting with microelectronics and electronics provides the means to do it.[7] Until now, the use of LED lighting for

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commercial production of crops has been limited to relatively small-scale experiments. This is mainly due to the high initial cost of the LED lighting installations in relation to conventional lighting. According to technology roadmaps, the costs of LED lighting will continue to decrease while energy efficiency will increase. These important development trends will facilitate and accelerate the use of LEDs in commercial horticultural applications. In addition to the well-known high energy efficiency potential, LEDs offer improved control possibilities over conventional lighting technologies such as high-pressure sodium (HPS) lamps. HPS lamps suffer from restricted controllability, long ignition times and dimming range limitations. Development of suitable control regimes connected with LED lighting can further reinforce energy savings. The full control of light intensity (i.e. dimming) is among one of the possibilities offered by LEDs. Furthermore, full-dimming of LEDs can be achieved without loss of operation reliability (e.g. reduction of the lifetime expectancy, abrupt failure). Nowadays, supplemental artificial lighting installations in greenhouses are commonly controlled based on outside global solar irradiance. Depending on weather conditions, physical obstacles, time of the day, sun inclination angle or period of the year, the spectrum, intensity and periodicity of daylight varies widely and continuously. Figure 1 shows examples of the daily variations of the global solar photosynthetic photon flux density (PPFD) at the experiment site during October measured at a 5-minute time interval.

To our knowledge, no investigation on the dynamic control of supplemental lighting intensity for optimised greenhouse cultivation of crop vegetables has been carried out. The aim of this study was to investigate the influence of continuous dynamic control of supplemental lighting intensity on electricity consumption of the lighting system and on final fresh weight of lettuce plants grown in greenhouse conditions. To achieve this objective, a dynamic lighting control (DLC) system was developed. The DLC regime was intended to instantaneously compensate for variations of daylight intensity below a defined control threshold level at the plant canopy. Additionally, this would avoid unnecessary supplemental lighting above the defined control threshold level with the aim at achieving energy savings. As reference, two additional lighting systems were used. The reference lighting systems were controlled according to conventional discontinuous on–off regime typically used in greenhouses.

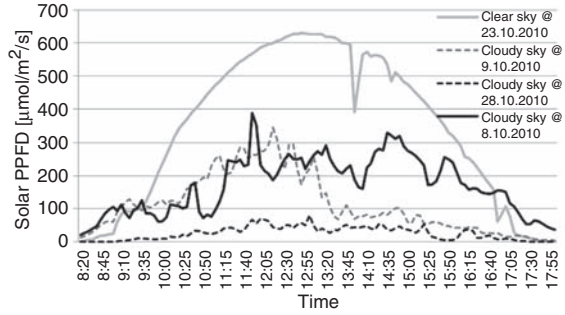

Figure 1 Daily variations of the global solar PPFD during clear and cloudy days in Viikki, Finland (60.228 N, 25.016 E) measured at 5-minute time intervals

2. Materials and methods

2.1. Plant material and growth conditions

A growth experiment was carried out in greenhouse conditions at Viikki, Finland (60.228 N, 25.016 E). Lettuce plants (*Lactuca sativa* L. cv. Frillice) were sown in pots (ø 6 cm) filled with peat substrate (B2S, Kekkilä, Finland) on 14 September 2010. After the first true leaves had just opened, the seedlings were placed in a hydroponic growing system under the three lighting treatments for 32 days between 27 September and 28 October 2010. In the hydroponic growing system, fertiliser solution (VihannesSuperEx 9-5-31 NPK, Kekkilä, Finland) with electric conductivity

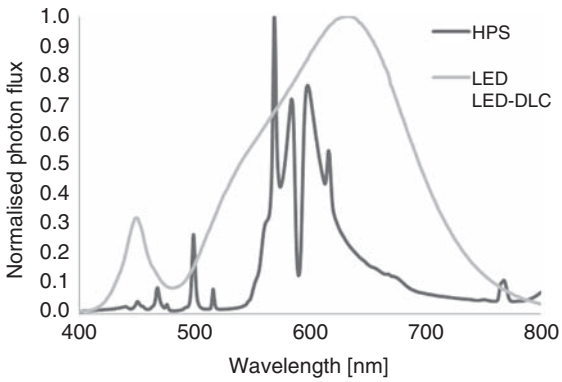

Figure 2 Normalised spectral photon flux distribution of the WW (2700 K) LED, WW (2700 K) LED–DLC and HPS lighting systems

of 1.6 mS/cm was used. White reflective plastics curtains were used to separate the lighting systems and to eliminate the lateral lighting interference between the systems. Analysis of variance was used to test the statistical significance of the fresh weight results.

2.2. Lighting systems

The lettuce plants were grown under three supplemental lighting systems (LED, LED–DLC and HPS). The LED and LED–DLC lighting systems were composed of 36 warm-white (WW) LED modules (13-W Light Line Source L-CM12/L-CM6, Citizen Electronics Co. Ltd, Japan) with correlated colour temperature of 2700 K. The viability of WW LEDs as artificial light source for lettuce growth was successfully tested in our previous research.[8] The HPS lighting system was composed of two high-bay luminaires (Cropmaker, Elektro-Valo Oy, Finland) equipped with 400-W HPS lamps (Lucalox^{TM} LU400/XO PSL/T/40, General Electric, USA). The measured relative spectral photon flux distribution curves of the lighting systems used are shown in Figure 2.

The photoperiod was set to 18 hours light (4:00 AM to 10:00 PM) and 6 hours dark (10:00 PM to 4:00 AM). The maximum average PPFD contribution due to supplemental lighting at the growth area was approximately 150 μmol/m^2/s. The average PPFD was determined based on measurements at 272 points uniformly distributed over the growth area of 1 m^2 at plant canopy height using a photosynthetically active radiation (PAR) sensor (LI-190SA, LI-COR, USA) connected to a light meter (LI-250A, LI-COR, USA). The measured average photon flux uniformity was approximately 80%. The measurement results of the average PPFD and lighting uniformities at growth areas are presented in Table 1.

Table 1 Maximum average supplemental PPFD contribution and uniformities of the LED, LED–DLC and HPS systems at the growth area

Lighting system	PPFD (μmol/m^2/s)	Lighting uniformity (%)
LED	152	83
LED–DLC	148	77
HPS	158	85

Notes: The results are the average of two series of measurements carried out at the start and end of the experiment.

2.3. Control regimes

The LED and HPS lighting systems were used as reference and were conventionally controlled using a discontinuous on–off regime. This discontinuous control regime was based on outside global solar irradiance. The LED and HPS lighting systems were switched off when the outside global solar irradiance exceeded the 270 W/m^2 threshold level. Due to starting limitations of HPS lamps and for reliability reasons, a time delay was included at turn-on and turn-off switching transitions. The regime was implemented using the greenhouse climate control installation (INTEGRO, PRIVA, the Netherlands).

The light output of the LED–DLC lighting system was continuously controlled in order to maintain a constant PPFD at the

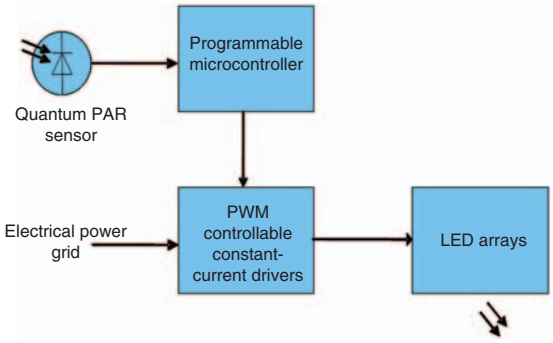

Figure 3 Simplified block diagram of the LED–DLC lighting system

plant canopy. Figure 3 shows a simplified block diagram of the LED–DLC circuit. The main components of the DLC circuit were the LED array, quantum PAR sensor installed on the top of the luminaire and a programmable microcontroller connected to the pulse width modulation signal input of the constant-current LED drivers. The microcontroller was programmed to proportionally respond to instantaneous variations of the daylight within 0 μmol/m^2/s to 150 μmol/m^2/s range at the plants' canopy height. The objective was to provide a constant PPFD of 150 μmol/m^2/s during the lighting period at the canopy level when the available solar PPFD was below 150 μmol/m^2/s. The electrical power demands of the lighting systems were measured and recorded at a time interval of 200 ms using a data acquisition unit (MX100, Yokogawa Electric Corporation, Japan).

3. Results and discussion

The fresh weight of the plants was measured at the end of the experiment and the respective averages were determined. The HPS plants had the higher fresh weight (219.8 g), followed by the LED (219.0 g) and LED–DLC plants (170.2 g). The average fresh weight of the LED–DLC plants was approximately 22% lower than that of LED and HPS plants. Differences in the fresh weight were highly significant ($p < 0.001$) according to an analysis of variance (SAS GLM procedure). According the Tukey's studentised range (honestly significant difference) test, the LED–DLC system differed from the HPS and LED systems, but there was no difference between LED and HPS. The HPS and LED systems operated under the same control regime and were equally effective in promoting the fresh weight accumulation of the plants.

The LED–DLC lighting system had the lowest total electricity consumption (206 kWh) at the end of the experiment followed by LED (256 kWh) and HPS (429 kWh). The LED–DLC system consumed 20% less electricity than the LED system without the DLC control (drivers excluded). However, there was no statistically significant difference between the LED and LED–DLC systems in terms of the ratio between the final average fresh weight of the plants and the total electricity consumption. The electricity consumption of the HPS system could not be directly compared to LED and LED–DLC systems due to the different optical (e.g. light spatial distribution profiles) and electrical characteristics as well as the small growth areas used. The plants' final average fresh weight, total electricity consumption of the lighting systems and their relation (i.e. g/kWh) are shown in Figure 4.

It is known that with a few exceptions, light quality mainly affects the morphological variables of plants, while productivity is mostly affected by changes in light quantity.[9] The light quantity can be quantified using the daily photosynthetic photon flux integral (PPFI) at the growth area. The PPFI on the LED and LED–DLC plants was estimated based on the daily electricity consumption due to its direct relation to the photon flux output of the lighting systems, as shown in Figure 5. For that, it was assumed that the daylight contribution to the total PPFI was identical in all lighting systems. As such, the

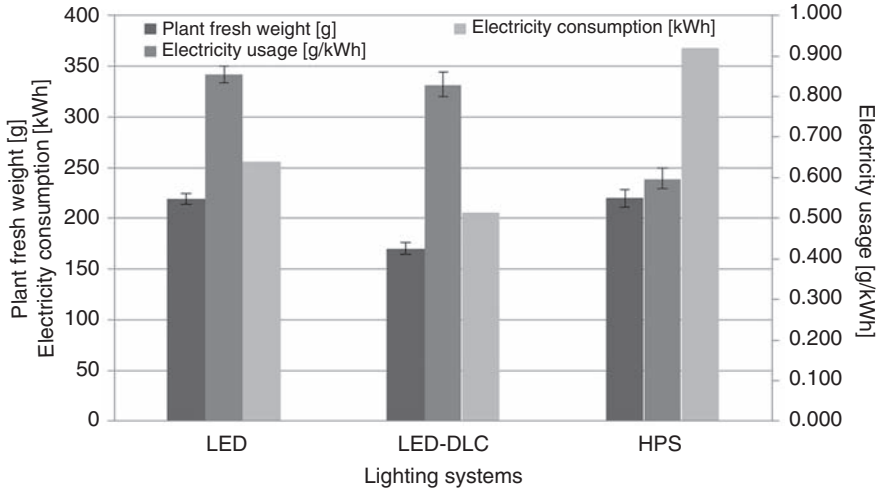

Figure 4 Final average fresh weight per plant (average ± standard error), total electricity consumption of the LED, LED–DLC and HPS lighting systems and ratio of fresh weight accumulation rate per unit of electrical energy consumed (average ± standard error)

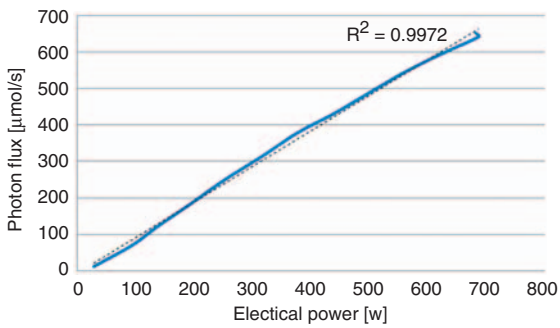

Figure 5 Measured and linear regression curves of total photon flux output emission as a function of the electrical power consumption due to dimming of the LED–DLC lighting system

20% lower energy consumption of the LED–DLC system in relation to the LED system has resulted in 20% lower PPFI, which closely matches the differences in the fresh weights of the plants (i.e. 22%). This suggests that the lower average fresh weight of the LED–DLC plants was caused by the smaller daily average PPFI. Moreover, fresh weight accumulation rate of lettuce plants benefits from increases on average PPFD up to 1000 µmol/m^2/s or on PPFI up to 17 mol/m^2/day.[10] For most crops, a 1% light increment results in 0.5% to 1% increase in harvestable product.[11] However, this is an average value, which depends on several factors. For instance, the relative effect of light on growth is greater at lower light levels, at higher CO_2 concentrations and at higher temperatures. Theoretically, light levels close to the saturation point of photosynthesis will allow for the maximum biomass accumulation or productivity due to the higher CO_2 fixation rate. The limits are set by the thermodynamic properties of the crop and its environment, namely the intercepted radiation by the plant canopy. According to the Monteith equation, the total dry matter content (biomass) at harvest is closely and linearly correlated with accumulated intercepted solar radiation.[12]

Future study should consider the dynamic control of supplemental lighting to be based on the daily PPFI in addition to the instantaneous PPFD. In that way, it can be expected that reductions on electricity consumption due to supplemental lighting are achieved together

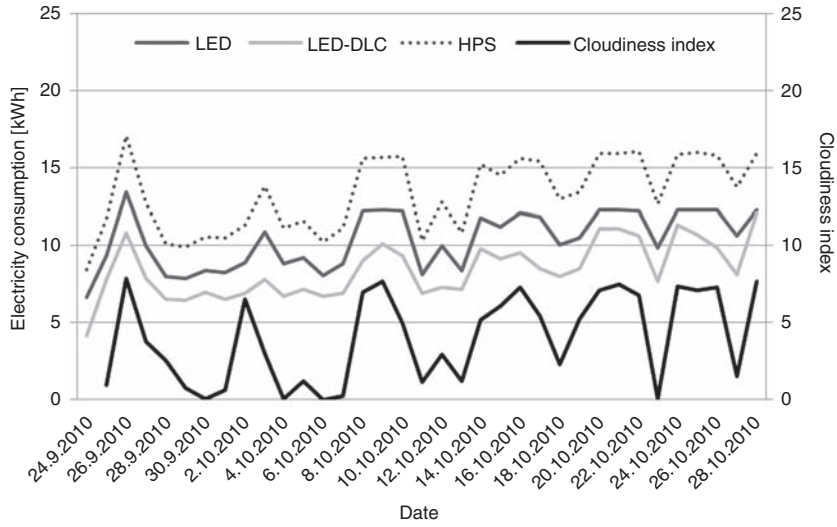

Figure 6 Variation of daily average cloudiness index (0–8) and electricity consumption of the LED, HPS and LED–DLC lighting systems during the experiment

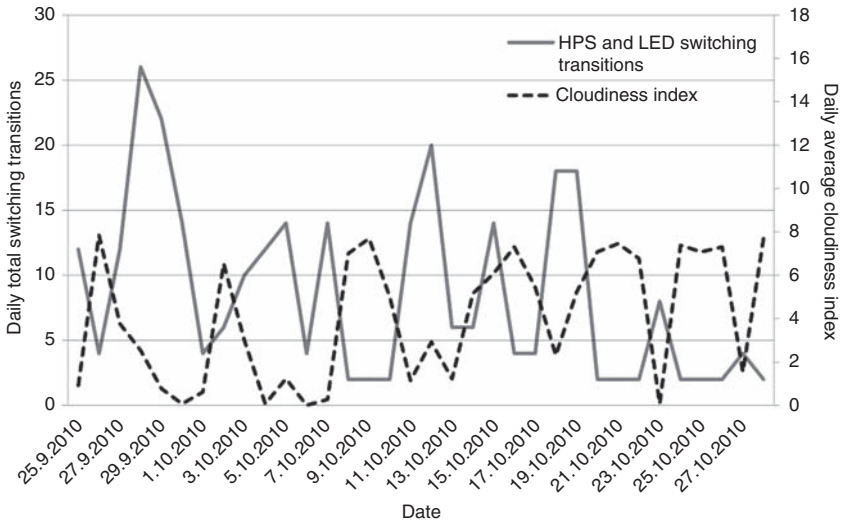

Figure 7 Variation of the daily total switching transitions and cloudiness indexes

with maximisation of crop productivity and photosynthesis efficiency. Conversely to cultivation in the open field, in the near future, the lighting conditions in closed environments are expected to be fully controllable. Although technically more challenging, further improvements on the DLC approach may include spectral optimisation considering the local, seasonal and daily variations of the daylight spectrum. LEDs are ideal candidates for the

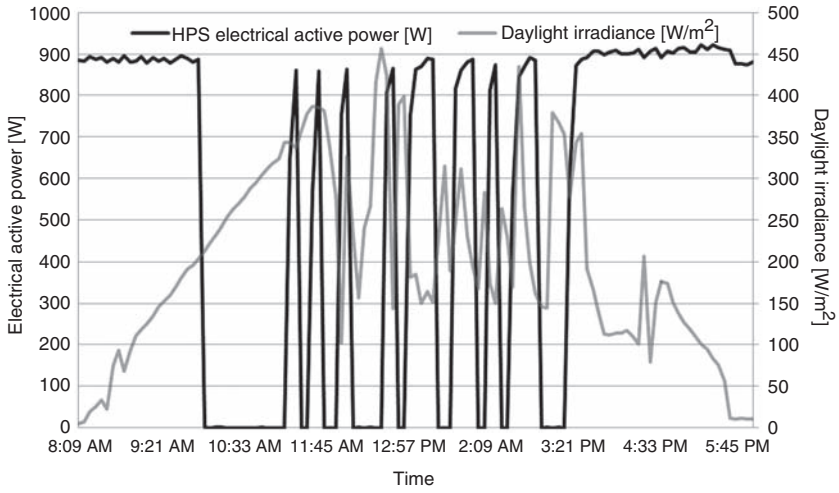

Figure 8 Effects of the fast variation of the daylight irradiance on the number of on–off switching transitions represented by the electrical active power curve of the HPS lighting system during a partially cloudy day on 12 October 2010

practical implementation of this kind of lighting environment.

The control of supplemental lighting in greenhouses is commonly based on the available global solar irradiance. The daylight availability (i.e. light intensity), among other factors, is influenced by the sky conditions and the period of the year. The cloudiness index, which is given by the ratio of the amount of diffuse to total solar radiation incident on the ground surface,[13,14] was used to assess the sky condition. In Figure 6 is shown the influence of sky conditions (i.e. cloudiness indexes) on electricity consumption of the lighting systems. Both control strategies showed a clear correlation between the daily average cloudiness index and the daily average electricity consumption. The electricity consumptions of the lighting systems were higher under cloudier skies (i.e. higher cloudiness indexes) than under clearer skies (i.e. lower cloudiness indexes). Coincidently, cloudy days are more frequent during the period of the year with the shortest day lengths and lowest solar irradiances[15] to sustain viable crop development. Therefore, this fact reinforces the need to maximise the use of the incoming solar radiation.

An inverse correlation was found between the cloudiness index and the number of switching transitions resulting from the on–off control regime used with HPS and LED lighting system, as shown in Figure 7. Switching transitions were more frequent during partially cloudy than on heavily cloudy days. This was due to the wide amplitude variations of the daylight intensity caused by temporary shadowing effects created by the clouds over the experiment site.

An example of the effects of a partially cloudy day on the number of switching transitions of the HPS system during the photoperiod is shown in Figure 8. During that specific day, 20 switching transitions using the HPS and LED lighting systems were detected. This gives an average of more than one switching transition per hour. At the end of the experiment, the final average daily switching transitions were nine, which correspond to 4.5 switching cycles. This switching cycle rate is more than twice that used to

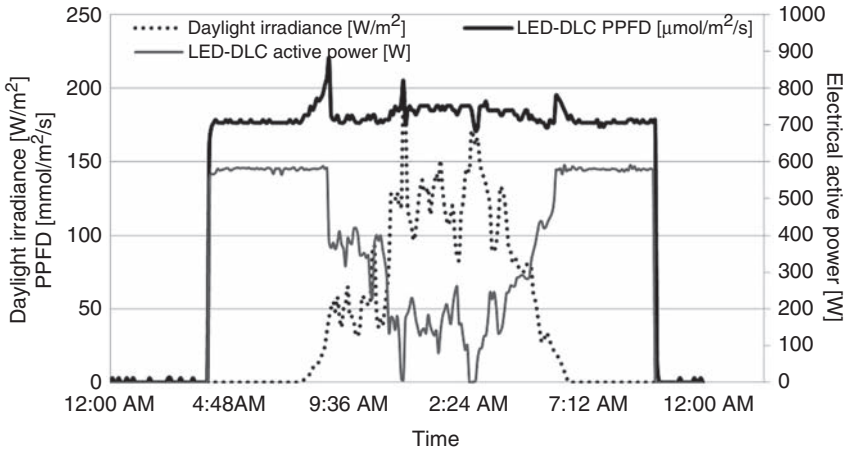

Figure 9 Variation of the exterior daylight irradiance, electrical active power consumption and PPFD at the central point of the LED–DLC growth area at canopy level during a cloudy day on 8 October 2010

Table 2 Lighting simulation results of HPS, WW LED, RCW LED and RB LED lighting installations in a 1000 m^2 area greenhouse building with an average PPFD and growth area of 150 μmol/m^2/s and 800 m^2, respectively

Lighting installation	Luminaire mounting height (m)	Installed power density (W/m^2)	Lighting uniformity (%)	Photon flux efficacy (μmol/J)
HPS	2.0	142	72.7	1.099
WW LED	0.5	244	92.8	0.619
RCW LED	0.5	207	92.8	0.729
RB LED	0.5	139	92.8	1.086

define the nominal lifetime of HPS lamps. It is likely that the stress caused by the frequent ignition on HPS lamps can negatively affect their lifetime, as happens to fluorescent lamps.[16] Moreover, premature failure of the lamp requires more frequent re-lamping, which will increase the maintenance costs and directly affect the system economics and the final production costs.

The DLC regime makes it possible to supplement the rapid variations of the daylight intensity and in that way maximise its usage. The use of a DLC regime in large greenhouses may give the possibility to create a less stressful lighting environment for workers by avoiding abrupt changes on lighting levels due to on–off switching. This situation is shown in Figure 9, where the variation of the PPFD at the central point of the LED–DLC growth area was maintained constant in spite of the continuous variations of daylight irradiance.

Lighting simulations of a large greenhouse installation were carried out in order to compare more accurately the energy consumption of HPS and LED systems based on required installed power density. The greenhouse structure was composed of a glass roof, double-sided acrylic walls and total horizontal area of 1000 m^2. The aimed average PPFD and growth area was 150 μmol/m^2/s and 800 m^2, respectively. Using the described greenhouse installation, several lighting designs were created and simulated. The best

simulation results for each lighting installation was selected based on the lowest installed power density achieved with a minimum lighting uniformity criteria at the growth area of 70%. Table 2 presents the mounting heights, installed power densities and the lighting uniformities obtained with simulations of the HPS and WW LEDs. Additionally, the results obtained for deep-red/cool-white (shortly RCW) and deep-red/blue (shortly RB) LED spectral combinations are also included for improved comparison. The RCW and RB LED systems used in the simulations have been tested in our previous experiments for cultivation of lettuce plants without daylight.[8]

The lighting simulation results showed that at system level, the energy efficiency of the HPS lighting installation (1.099 μmol/J) was approximately 44% more energy-efficient than the WW LED solution (0.619 μmol/J). The results showed also that RB was the most energy-efficient LED combination. However, our previous studies suggest that the RB spectral quality might not be the ultimate LED solution to promote fresh weight accumulation of lettuce plants without daylight. The energy efficiency of the RB LED installation (1.086 μmol/J) was similar to HPS installation (1.099 μmol/J).

4. Concluding remarks

Improvement in plant productivity and conversion efficiency of the absorbed radiation into edible biomass may be achievable using appropriate control of light quality, quantity and periodicity. In this paper, the effects of continuous and discontinuous control of supplemental lighting intensity on the fresh weight accumulation of lettuce plants and on electricity consumption of HPS and LED lighting systems were evaluated. One of the advantages of the DLC over the conventional on–off regime is the fast response time to the variations of daylight intensity. Additionally, the continuous operation mode of the DLC regime allows for a more exact compensation for lack of daylight than the on–off control. This feature enhances the electrical power-saving potential of the lighting installation in relation to conventional on–off control. The use of continuous lighting control (LED–DLC) resulted in 20% reduction of the electricity consumption in comparison to similar lighting system operated under discontinuous on–off regime (LED). However, the LED and LED–DLC system performed similarly in terms of average fresh weight of the plants accumulating per unit of electrical energy consumed. These results indicate that further optimisation of the DLC regime is needed in order to reduce electricity consumption, without hindering the productivity of the plants.

In this study, LEDs were indispensable to implement the DLC regime without loss of system reliability. The operation reliability (e.g. lifetime) of conventional high-intensity discharge lamps, such as HPS lamps, can be influenced by the number of switching cycles. The on–off regime used to control the HPS and the LED lighting system had a daily average number of switching transitions twice as high as that used to define the lifetime of HPS lamps. The stress caused by the frequent ignition of HPS lamps can accelerate the depreciation of light output due to the faster electrode deterioration, blackening of the arc tube or changes in ballast electrical performance. Therefore the on–off control regime used in our experiment is not recommended to be used in practical applications for the sake of operation reliability and minimisation of maintenance costs due to re-lamping.

The use of LEDs in horticulture lighting offers novel possibilities of control compared to incumbent lighting technologies. These possibilities include the full digital control of the light quantity, in order to optimise the crop productivity. The digitalisation of lighting allows for the development of more intelligent supplemental lighting systems, which may contribute to a more sustainable year-round production of food in protected and controlled

environments. LED technology can contribute to a successful response to these increasingly urgent global needs. In this paper, WW LEDs were confirmed as a viable supplemental photosynthetic light source for lettuce growth. However, the utilisation of WW LEDs in commercial greenhouses will depend on future developments of the LED technology and its related costs. Nevertheless, according to our simulation results, energy-efficient retrofitting of conventional HPS systems for lettuce cultivation will require installed efficacies above 1.1 μmol/J. The RB LED combination was still the most energy-efficient LED solution. However, further studies have to be carried out in order to fully validate the energy efficiency of different LED spectra taking into consideration the productivity and economic viability of retrofitting existing lighting installations with LEDs.

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Glossary

#

#30 sieve - A strainer that the contents of the kick net is emptied into to remove unwanted debris. The sample material remaining is placed in whirlpacks.

A

Attached Algae - Algae that has grown attached to a solid object or organism.

B

Bank Full Width - Width of a stream bank at full flood stage.

Bank Stability - The ability of a stream bank to counteract erosion or gravitational forces.

Baseline Sample - A sample of the quality of water when the body of water is at a normal or resting state. This can be used later on as a comparison to samples that are taken during or after storms.

Benthic Macroinvertebrates - Organisms that do not have spines, and are generally small, but visible without a microscope. They are abundant near bodies of water and surrounding ecosystems, and usually live in water at some stage of their lives.

Berm - A level space, shelf, or raised barrier separating two areas. These are constructed to control runoff and direct flow.

Bioassessment - (or Biological Assessment) A method of assessing aquatic conditions by surveying biological organisms, such as macroinvertebretes, fish, or plants.

Biological Sampling - Conducting a survey of biological organisms used for beneficial research.

C

Canopy Cover - The amount of sky covered by trees and vegetation over a stream bank.

Channel - In the context of this research, refers to the physical confinement of a stream that the water flows through, consisting of the stream bed and banks.

Channelized - Is the straightening and modification of a river corridor as a way to control the water. However, it is difficult to maintain a straight river, as the water tends to erode along the banks to return to a natural winding river.

Channel Sinuosity - A streams natural ability to bend and wind, an important characteristic of rivers to divert high flows and carry/deposit sediment.

Chemical Constituents - The amount of oil, alcohols, aldehydes, esters, ketones, lactones, phenols and terpenes in a water sample.

Cross sectional area - The area of a slice of river, perpendicular to flow; used to help determine stream velocity.

D

Deposition - The accumulation of material out of the water and onto the stream bed.

Didymo (Rock Snot) - A type of freshwater algae that is a nuisance when it blooms, creating thick, brown mats on the streambed. It is found in certain areas of Vermont, therefore waders and nets are decontaminated after use to avoid spreading it.

Discharge (flow) - The rate that a volume of water (and its associated suspended solids, dissolved chemicals, and biological materials) flows over a specific time. Usually provide in cubic feet per second.

Dissolved Oxygen - A relative measure of the amount of oxygen that is dissolved or carried in the stream water.

Dredging - The scooping and removal of sediment etc. from the bottom of a stream.

E

Ecological Integrity - The abundance and diversity of organisms at all levels, and the ecological patterns, processes, and structural attributes responsible for that biological diversity and for ecosystem resilience.

Eddies - The swirling of stream water , usually downstream and past a barrier.

Embeddedness - How much of an object is submerged into the substrate under the water.

Epifaunal - Animals that live on the surface of substrate, such as rocks, pilings, vegetation, or the streambed itself.

Ethanol - A form of alcohol that is used to clean lab materials, as well as to preserve insect specimens.

F

Floating Algae - Algae that is not attached to anything, typically refers to mats of algae that have accumulated and are growing together on the water's surface.

Free Floating Algae- Algae that is not attached to anything, such as duckweed.

H

Habitat Assessment Data Sheet - A field sheet used to determine habitat parameters of a stream site.

Habitat Equality - The balance of things within a given habitat.

Headwaters - A tributary stream of a river close to or forming part of its source.

I

iButton - A sensor that measures and records temperature. It works by transferring data in and out of the sensor when it is connected by a USB device.

iButton Capsule - A capsule that protects the iButton from environmental conditions such as temperature, moisture, pressure, and solvents, and allows the iButton to be securely mounted in a stream environment.

Infiltration - The movement of water into and through soil.

In Situ Measurements - Standard parameters that can be taken on the stream site with a water quality instrument.

J

K

Kick net - A net that is placed, with the opening facing upstream, into the riverbed with the motive of capturing benthic macroinvertebrates. While holding the net stable again the stream bottom, the researcher kicks and stirs up the sediment in front of the net, capturing any organisms living in and around the area.

L

Large Woody Debris - Large pieces of wood found in streams, that acts as important habitat for aquatic organisms.

M

Macroinvertebrates - see *Benthic Macroinvertebrates*

Macroinvertebrate Data Sheet - A sheet which records the conditions of the stream. This includes pebble count, canopy cover, temperature, water velocity, pH, and width data. It is used to record Macroinvertebrate collecting locations.

Macroinvertebrate Habitat Data Sheet - A field sheet that focuses on macroinvertebrates. It includes the pebble count.

N

Nitrogen - An odorless and colorless element that makes up about 78% of the earth's atmosphere and is necessary for life to exist. Too much dissolved nitrogen in a water source can lead to eutrophication.

NOAA - Stands for the National Oceanic and Atmospheric Administration, a Department of Commerce agency that maps out oceans, predicts climate changes, provides weather and natural disaster reports, and helps conserve oceanic resources.

O

One-Wire Viewer - iButton temperature sensor software for your computer. A Java demonstration application for iButton that features from your PC.

Orthophosphate - A lone phosphate molecule, a phosphorus atom connected to four oxygen atoms. Orthophosphate is directly taken up by algae .

Outfalls - The place where a river, drain, or sewer empties into the sea, a river, or a lake.

P

Pebble Count - The tallying of 100 or more random sediment samples, measured by walking up and downstream in a zig-zag pattern and selecting random points to measure along the way.

Phosphorus - A solid, nonmetal element (P) that is necessary for life and typically exists in nature as a phosphate molecule (PO_4). Inorganic and organic phosphorus can be dissolved or suspended in water and too much phosphorus in a water source can lead to eutrophication.

Physical Characterization - The physical things that describe the stream.

Physical Constituents - The physical makeup of a stream.

Pools - Deep parts of streams that typically occur after riffles.

Poison ivy - A toxic, flowering plant with three leaves that is common locally. It is known for irritating skin that comes in contact with it.

Poison parsnip - A common, local, flowering plant with yellow flowers. Can be an irritant if the inner sap is exposed and comes in contact with skin.

Q

Quaternary Ammonium Disinfectant - A combination of water and quaternary ammonium (QUAT) that is used to sanitize waders after using them; ensuring that nothing harmful is transmitted when they are transported.

R

RACC - Stands for Research on Adaptation to Climate Change that aims to answer the following overarching question: How will the interaction of climate change and land use alter hydrological processes and nutrient transport from the landscape, internal processing and eutrophic state within the lake and what are the implications for adaptive management strategies?

Replicate Number - The numbering of multiple samples for the purpose of organization.

Riffles - A rocky or shallow part of a stream or river with rough water that is typically high in dissolved oxygen.

Riparian Zone - The area between land and river or stream.

Riprap - Loose stone used to form a foundation for a breakwater or other structure.

Rooted Emergent - Refers to a plant that is rooted in sediment below a body of water, such as cattails.

Rooted Floating - Refers to an aquatic plant that is rooted below a body of water that floats to the top, such as lilies.

Rooted Submergent - Sediment rooted under a body of water that does not stick out, such as water milfoil.

S

Sample ID Number - Located at the top of the Macroinvertebrate Data Sheet, this ID Number consists of yy/mm/dd and the Replicate number.

Sensor Field Data Sheet - To be completed at each field site; records temperature and sage sensor data.

Snag - In aquatic systems, this refers to trees and branches that have fallen into the stream.

Stage Sensor (HOBO Water Level Logger) - A battery powered device that is used by RACC which measures stage or water level of fresh water streams.

Stream Gradient - The slope of a stream. How to know if your stream site is high or low gradient:

1. Determine the stream type using this chart below.
    a. Is your stream site confined by valley walls?
    b. What is the general valley slope of your site?
        i. Valley width is important because it is an indicator of how confined the stream is and whether it will have access to a floodplain at different flood levels. To determine valley width differences look for relative changes in the distance between toes of opposing valley walls. The toe of a valley wall can be identified as the bottom of the more steeply sloped portion of the valley.
        ii. If your site is unconfined by valley walls and <2% slope (think fairly flat, not down a steep hill, the water has access to a floodplain when it rains, etc) you'd classify it as a type C stream.
        iii. If your site has a steeper slope and valley walls that confine the stream (does it have room to meander or change course?), you'd classify it as a type A stream.

Table 2.2 Phase 1 – Reference Stream Typing Chart

Reference Stream Type	Confinement (Valley Type)	Valley Slope
A	Narrowly confined (NC)	Very Steep > 6.5 %
A	Confined (NC)	Very Steep 4.0 - 6.5 %
B	Confined or Semi-confined (NC, SC)	Steep 3.0 - 4.0 %
B	Confined or Semi-confined or Narrow (NC, SC, NW)	Mod.- Steep 2.0 – 3.0 %
C or E	Unconfined (NW, BD, VB)	Mod.- Gentle < 2.0 %
D	Unconfined (NW, BD, VB)	Mod.- Gentle < 4.0 %

2. Once you know what your stream type is, you can use the table below to determine if your site is high or low gradient.
    a. If your site is a type C stream, think about the substrate. Is the stream mostly gravel, cobble, or boulders? If so, you're in a high gradient stream.
    b. If your site is a type C stream but has mostly sand or fine gravel substrate, your site is a low gradient stream.

When to use high gradient RHA field form	**When to use low gradient RHA field form**
- reference stream type is A or B	- reference stream type is E
- reference stream type is C characterized by riffle/pool bed features and a dominant substrate size of gravel or larger	- reference stream type is C with ripple/dune or riffle/pool bed features and dominant substrate size is fine gravel, sand or smaller

For example, our training week field sites are classified below:

Potash Brook:

Stream Type: C
Substrate: Gravel and larger (cobbles)
Classification: High Gradient

Allen Brook:

Stream Type: C
Substrate: Sand and silt
Classification: Low Gradient

Munroe Brook:
Stream Type: B
Classification: High Gradient

Indian Brook (by Essex High School):
Stream Type: C
Substrate: Sand and silt
Classification: Low Gradient

Indian Brook (by Mill Pond):
Stream Type: C
Substrate: Gravel and larger (cobbles)
Classification: High Gradient

Stream Reach - A section of stream having relatively uniform physical attributes, such as confinement, valley slope, sinuosity, dominant bed material, sediment regime, tributary influence, and bed form. Reach determinations do not take into account human disturbances, but rather are based on variables related to valley setting, stream morphology, and their inherent fluvial processes.

Stream Site Code - A code given to any stream being tested so it can be easily identified in a lab.

Stream Site General Assessment Data Sheet - A field sheet that is filled out annually for a stream site. It provides general information about the location, surrounding area, and watershed features (such as a nearby dam or bridge).

Stream Stage - The height (typically in ft) of water from an established point, typically from stream bottom to surface. Often maintained by the USGS and can be measured in a variety of ways.

Substrate - Represents the variety of matieral that is present in the stream, ranging from clay and gravel, to boulder and bedrock, and includes woody debris. Refer to the following table for sizes:

Clay/Silt/Sand	< 0.004-2.0	Fine, granular pieces of sediment measuring under 2.0 cm
Gravel	2.0-16	Small rocks measuring 16 cm or less
Course gravel	16-64	Larger (softball size or bigger) rocks that are smaller than 64 cm
Cobble	64-256	Chunks of rock that are not large enough to be boulders but are still noticeably sizeable.
Boulder	>256	Large Rock measuring above 256 cm, tall (relative to surrounding sediment) and above the bedrock.
Bedrock	---	Solid rock, providing a base layer over which there are other sediments.

T

Thalweg - A line connecting the lowest or deepest points of successive cross-sections along the course of a valley or river. This where the largest volume of water flows within the stream.

Ticks - Small, parasitic (blood sucking) organisms found locally. May transmit diseases including Lyme disease. Following time in the field, researchers should check for ticks on clothing and exposed skin.

Total Suspended Solids (TSS) - The total amount of suspended solids in a sample of water; listed as a pollutant in the US Clean Water Act and is therefore measured as a water quality indication. Includes mostly sediment and algae.

Total phosphorus (test) - A test that measures all phosphorus forms, such as orthophosphate, condensed phosphate, and organic phosphate, in a given sample of water.

Tributaries - A river or stream flowing into a larger river or lake.

Turbidity - The cloudiness of water caused by small particles.

U

USB adaptor - An adapter that allows information to be directed between the iButton and a computer via a USB port.

USEPA - Stands for the United States Environment Protection Agency, a US federal agency that protects human health and the environment through enforcing regulations and laws passed by Congress.

USGS - Stands for the US Geological Survey, a US federal agency that studies the landscape of the United States and its natural resources and hazards.

V

Valley Slope - While you don't need to calculate the actual valley slope, it is good to know how the calculation is done.

Example – Calculating Valley Slope

1140 ft	upstream elevation
- 1000 ft.	downstream elevation
140 ft	change in elevation

$$\frac{\text{difference in elevation (ft.)}}{\text{length of valley (ft.)}} = \frac{140}{4{,}000} = 0.035 \times 100 = 3.5\ \%\ \text{valley slope}$$

Velocity - In this context, the speed at which the water is flowing downstream.

W

Water Quality Assessment - An evaluation of the conditions of a body of water. Specifically, biologically and chemically assessing and analyzing components such as flow, pH, TSS and nutrients of the body of water.

Water Quality Monitoring - Sampling and analysis of water constituents and conditions such as pollutants, natural components, dissolved chemicals, bacteria, etc. to know the base condition and target changes that may occur.

Water Quality Parameters - The general measurements of water that are healthy.

Watershed - An area or ridge of land that separates waters flowing to different rivers, basins, or seas.

Wetted Width - The width of the water in a stream bank.

Whirlpacks - Small bags that captured specimen are placed in after being captured in the kick net. Following this step, add ethanol for preservation.

X

Y

Z