

The University of Damascus

Faculty of Agriculture - Swaidaa branch

English Module, Fifth year, Lectures:1 , 2

FINAL-YEAR PROJECT REPORT WRITING GUIDELINES

This guide is meant to help you produce a good final year project or dissertation. A good report is one that presents your project work concisely and effectively. It should contain various materials relevant to the work you have undertaken in respect of your project; it should be organised into a logical framework; and it should be supported by written material that follows well-established academic conventions in a consistent fashion.

The purpose of the project is to integrate various aspects of the taught material and to demonstrate your (academic) research skills and your (professional) analysis, design and implementation skills. It gives you the opportunity to conduct in-depth work on a substantial problem to show individual creativity and originality, to apply where appropriate knowledge, skills and techniques taught throughout the degree programme to further oral and written communication skills, and to practise investigative, problem-solving, management and other transferable skills.

When you choose a project, you should do so carefully, to reflect the focus of the degree programme you are enrolled in, your personal interests (the project needs to keep you interested for the whole the academic year) and the ability of the academic staff to support you throughout your project. Your project supervisor will guide you on what it is reasonable to expect a project in your chosen topic to deliver.

Sections that you need to include in your report are:

- | | | |
|------------------------|-----------------|--------------------------|
| 1- Title & Authors | 2- Abstract | 3- Acknowledgements |
| 4- Table of contents | 5- Introduction | 6- Experimental |
| techniques and methods | 7- Results | 8- Discussion |
| 9- Conclusions | 10- References | 11- Appendices (if used) |

1- Title: This page should contain the following information: Title, Full name of author, Degree that it contributes towards, Industrial or other collaboration if appropriate, Month and year of submission. Note that the title page should not show a page number.

2- Abstract: The second page should consist only of the abstract. The idea of the abstract is to provide a brief summary of the report. The reader should be able to pick up from the abstract what the abstract entailed, how it was undertaken and an indication of what was found out. An abstract should not review the report, but should rather act as a sampler of the contents of the report. Typically the abstract should be less than 200 words. It should be not more than one page in length.

It should allow the reader who is unfamiliar with the work to gain a swift and accurate impression of what the project is about, how it arose and what has been achieved. Importantly, abstract should enthuse the readers about the project to encourage them to read the whole report.

In the abstract you should:

- ◆ State main objectives – What did you investigate and why?
- ◆ Describe methods – What did you do?
- ◆ Summarize important results – What did you find out?
- ◆ State main conclusions – what do your results mean? .

Do not include, references, figures, etc., information in the report, background information. Extract key points and condense material.

3- Acknowledgements: It is normal to thank those who have given help and support (typically your supervisor). Keep acknowledgements short and business-like.

4- Contents List: This should give a complete list of what the report contains starting with the abstract (the title page is not included in the contents list). This should comprise a table of contents, indicating the page numbers of the different sections.

List of Tables/Figures: If the report contains figures or tables a list of these should be provided. The list should give the table or figure number, the title of the table or figure and the page number. If only a few tables and figures are present, they may be treated on one page.

Remember that all figures and tables used must be referred to in the text. For example “The class diagram shown in Figure 2.1”

5- Introduction: The Introduction “introduces” the reader to your topic or subject (why are you writing this article?). Consequently, you need to arrange the information from general to more specific. It is also important to give your reader a historical context so that they can understand the significance of your article. The best way is to refer to previous publications.

The Introduction section should answer two questions: “What?” and “So what?” What is the paper about, and why should the reader care?. An introduction should inform the reader as to what the report is about and motivate the reader to continue reading. The main purpose of the introduction is to provide the typical reader with information that they cannot be expected to know, but which they will need to know in order to fully understand and appreciate the rest of the report. It should explain why the project is addressing the problem described in the report, indicate an awareness of other work relevant to this problem and show clearly that the problem has not been solved by anyone else..

Introduction should:

- ◆ Describe the problem investigated.
- ◆ Summarize relevant research to provide context, key terms, and concept so the reader can understand the experiment.
- ◆ Review relevant past research to provide rational for your work.
- ◆ Briefly describe your research – design, research, hypothesis, etc.
- ◆ Move from general to specific – relate problems in the real world to your research.
- ◆ Make clear links between the problem and the solution. Be selective in choosing studies to cite.

Long descriptions of details are to be avoided and references to suitable sources of detailed information should be given instead. Your background section should end with a clear statement of the research problem (your project is trying to answer). Some common pitfalls in writing an introduction include unnecessary background information (telling the reader what they already know or what they do not need to know), exaggerating the importance of the work, or failing to make clear what research questions this paper is trying to answer.

6- Experimental techniques and methods: For experimental work, give sufficient detail about your materials and methods so that other experienced workers can repeat your work and obtain comparable results. When using a standard method, cite the appropriate literature and give only the details needed. Explain how you studied the problem. Depending on the kind of data, this section may contain subsections on experimental details, materials used, data collection/sources, analytical or statistical techniques employed, study area, etc. Provide all background data, equations, and formulas if they are necessary to the arguments. Include charts, maps or tables if they aid clarity or brevity. Do not include results, extraneous details, or treating the method as a chronological history of what happened.

The methods must explain:

- ◆ How you studied the problem and what you used – materials, subjects and equipment.
- ◆ How you performed the research – methods and procedure.
- ◆ Provide enough detail for replication of your work. Order procedures chronologically. Use past tense to describe what you did. Don't mix results with procedure.

Methods section should be sufficiently detailed so that an independent researcher working in the same field could reproduce the results to allow validation of the conclusions. Often, this does not require explicit step-by-step instructions but rather references to prior publications that provide such details. A carefully written method section should not only describe what was done and how it was done, but it should justify the experimental design. Statistical considerations, such as sampling plans and analysis methods used, should be explained.

Data Collection and Data Analysis: Data can be collected using various methods: survey questionnaires, interviews, field observations, experiments, or even secondary data from works of other researchers. The choice of a data collection method, or a combination of methods, to be used depends very much on the research methodology adopted. The student must be able to provide a clear presentation of data collected.

To improve your data analysis skills and simplify your decisions, execute these five steps in your data analysis process:

Step 1: Define Your Questions: you must begin with the right question(s). Questions should be measurable, clear and concise. Design your questions to either qualify or disqualify potential solutions to your specific problem or opportunity.

Step 2: Set Clear Measurement Priorities: This step breaks down into two sub-steps: A) Decide what to measure, and B) Decide how to measure it.

A) Decide What To Measure: consider what kind of data you'd need to answer your key question. Finally, in your decision on what to measure, be sure to include any reasonable objections any stakeholders might have.

B) Decide How To Measure It: Thinking about how you measure your data is just as important, especially before the data collection phase, because your measuring process either backs up or discredits your analysis later on. Key questions to ask for this step include:

1- What is your time frame? 2- What is your unit of measure? 3- What factors should be included?

Step 3: Collect Data: With your question clearly defined and your measurement priorities set, now it's time to collect your data. Keep your collected data organized in a log with collection dates and add any source notes.

Step 4: Analyze Data: After you've collected the right data to answer your question from Step 1, it's time for deeper data analysis. Begin by manipulating your data in a number of different ways, such as plotting it out and finding correlations or by creating a pivot table in Excel. A pivot table lets you sort and filter data by different variables and lets you calculate the mean, maximum, minimum and standard deviation of your data.

During this step, data analysis tools and software are extremely helpful. Visio, SPSS, R, Minitab and Stata are all good software packages for advanced statistical data analysis. However, in most cases, nothing quite compares to Microsoft Excel in terms of decision-making tools.

Step 5: Interpret Results: After analyzing your data and possibly conducting further research, it's finally time to interpret your results. As you interpret your analysis, keep in mind that you

cannot ever prove a hypothesis true: rather, you can only fail to reject the hypothesis. Meaning that no matter how much data you collect, chance could always interfere with your results.

By following these five steps in your data analysis process, you make better decisions for your question because your choices are backed by data that has been robustly collected and analyzed.

7- Results: In this section you literally give the results of the analysis, but you don't interpret them. That occurs either in the next section, Discussion. **Present** your results in a logical sequence, highlighting what is important and how the data you obtained have been analysed to provide the results you discuss. You should discuss what you infer from the data. You need to adopt a critical approach. **Make** sure that all diagrams, graphs etc. are properly labelled and have a caption. A neat hand drawn diagram is preferable to a poorly made computer diagram, or a poor resolution image copied from the web.

Results explain your actual findings, using subheadings to divide the section into logical parts, with the text addressing the study aims. Link your writing to figures and tables as you present the results. The achieved results are often organized into a series of Tables and/or Figures to present your key finding in a logical order. Important negative results should be reported, too. Mention any uncertainty in measurement or calculation, and use an appropriate number of decimal places to reflect it. Make comments on the results as they are presented, but save broader generalizations and conclusions for later.

Figures and Tables: Because “a picture is worth a thousand words”, you should plan to illustrate your report with maps, images, etc. Any image is called a figure and each is given a separate figure number. Be sure to refer to each figure in the text, so your reader will know what image you are referring to. **Tables** allow you to present a great deal of information in a non-narrative format, i.e., tables are lists. However, a table should not repeat all the information given in text. Each table should have a caption. As with Figures, Tables numerical and should be referred to in the text. A table should be created using the Table menu, not using the tab or space bar.

8- Discussion: The purpose of the discussion is to interpret and compare the results. Be objective: point out the features and limitations of the work. Relate your results to current knowledge in the field and to your original purpose in undertaking the project. Briefly state the logical implications of your result. Suggest further study or applications if warranted. Say what

you actually found, not what you hoped to find. Do not repeat information given elsewhere in the project. The discussion explains the meaning of the results or why the results differ from what others have previously reported.

9- Conclusion: The purpose of the Conclusion section is to restate the study aims and key questions and summarize the findings. Keep this section brief and to the point. Do not repeat discussion points or include irrelevant material. The conclusions should be based on the evidence presented. If the conclusion is short (one or two sentences), then you can just add it to the end of the Main Body rather than make it a distinct section. The Conclusion is a good place to set your results in a bigger picture, which might help the reader understand the significance of your article and also a good place to recommend future research, perhaps in the form of a hypothesis. Regardless of what you include in the Conclusion, the last sentence should convey closure so that the reader recognizes “The End” without you having to write those words.

Notes: Pages, diagrams, references and tables must all be numbered. The entire report must be submitted on A4 size paper. The print quality should be dark and clear. Left margin 25mm. Top, bottom and right margins 20mm. Right and left margins should be justified (giving a straight edge to the text on both sides). Use a Times New Roman or similar style font, point size 12 for most of the document. A header should be placed at the top of each page apart from the title page. The text of the document should be double-spaced.

Sections and subsections should be in bold, they are used to organise the document into cohesive chunks for the reader. Give them meaningful headings. Never subdivide beyond three levels e.g. 3.1.1.2 is too much. Below is an example of the numbering system:

1 Chapter 1, 1.1 Section 1 of Chapter 1, 1.1.1 Sub-Section 1 of Section 1

A Division A of Sub-section 1: i.) Sub-Division i.) of Division A, ii.) Sub-Division ii.) of Division A

10- References.

11- Appendices: These should be used for reference material, such as selected pieces of research data and other information which is too bulky, or would detract from the flow of the text, if included in the main body of the document. Try to make reference material that will be consulted from many different places in the document and appendix. Otherwise, put the information into a figure or table and keep it close to the text that refers to it.

Citing & Referencing: Harvard Style

There are many styles that can be used for referencing. When you are given coursework or dissertation guidelines, check which style of referencing your lecturer or department asks you to use. If you don't check, and you use a style that is not the one stated in your guidelines, you could find you lose marks. This guide introduces you to the Harvard referencing style, which uses an 'author-date' approach. If your lecturer or department does not ask you to use any particular style, we would recommend using Harvard. It's easy to learn, simple to use, and when you get stuck, there is lots of advice available to help you out.

When you begin your research for any piece of work, it is important that you record the details of all the information you find. You will need these details to provide accurate references, and to enable you to locate the information again at a later date, should it be necessary to do so. Section 6 of this guide will help you identify what information you need, regardless of which referencing style you choose to use.

1. WHAT IS REFERENCING?

It is a method used to demonstrate to your readers that you have conducted a thorough and appropriate literature search, and reading. Equally, referencing is an acknowledgement that you have used the ideas and written material belonging to other authors in your own work.

As with all referencing styles, there are two parts: citing, and the reference list.

2. WHY SHOULD I REFERENCE?

Referencing is crucial to you to carry out successful research, and crucial to your readers so they can see how you did your research. Knowing why you need to reference means you will understand why it is important that you know how to reference.

1. Accurate referencing is a key component of good academic practice and enhances the presentation of your work: it shows that your writing is based on knowledge and informed by appropriate academic reading.
2. You will ensure that anyone reading your work can trace the sources you have used in the development of your work, and give you credit for your research efforts and quality.

3. If you do not acknowledge another person's work or ideas, you could be accused of plagiarism.

Plus your lecturers are very keen to see good reference lists. Impress them with the quality of the information you use, and your references, and you will get even better marks.

3. WHAT SHOULD I REFERENCE?

You should include a reference for all the sources of information that you use when writing or creating a piece of your own work.

4. WHAT IS A CITATION?

When you use another person's work in your own work, either by referring to their ideas, or by including a direct quotation, you must acknowledge this in the text of your work. This acknowledgement is called a citation.

When you are using the Harvard style, your citation should include:

1. The author or editor of the cited work
2. The year of publication of the cited work

5. HOW DO I WRITE CITATIONS USING THE HARVARD STYLE?

There are a number of rules relating to citations depending on the number of authors of a work, and if you are citing a quotation.

Citing one author

A recent study investigated the effectiveness of using Google Scholar to find medical research (Henderson, 2005). **Or:** Henderson (2005) has investigated the effectiveness of Google Scholar in finding medical research.

Citing two or three authors

If the work has two or three authors, include all names in your citation.

Recent research indicates that the number of duplicate papers being published is increasing (Arrami & Garner, 2008).

Evidence shows that providing virtual laboratory exercises as well as practical laboratory experience enhances the learning process (Barros, Read & Verdejo, 2008).

Citing four or more authors

If the work has four or more authors/editors the abbreviation 'et al' should be used after the first author's name. It is also acceptable to use 'et al' after the first author if the work has three authors.

Social acceptance of carbon capture and storage is necessary for the introduction of technologies (van Alphen et al, 2007).

6. HOW DO I WRITE A REFERENCE?

To write your own references you need different bits of information about each item that you read when you are researching a piece of work. These bits of information are called 'bibliographic' information.

For all types of references the key bits of information you need to start with are:

1. Author or editor
2. Date of publication/broadcast/recording
3. Title of the item

This will form the basis of each reference you have to write. You may find that some items are not as straightforward as others, so be aware of the following:

1. Author/editor: This means the primary (main) person who produced the item you are using.

If you are using a website or web page, and there isn't an author, you can use what is called a 'corporate author'. This will usually be the name of the organisation or company to whom the website or web page belongs.

2. Date of publication/broadcast/recording: This means the date the item was produced. It is usually a year, but if you are using a newspaper article, an email, or a television recording, you will have to include a full date (day/month/year) in your reference.

3. Title of the item: This means the primary (main) title of the item you are using. That sounds very obvious, but have a look at a web page and try to work out what the main title is. We would advise common sense in this situation – you have to identify the key piece of information that describes what you have used, and will allow the reader of your work to identify that information.

Depending on the type of material you want to reference you will also need other bits of information, such as:

- Name of publisher
- Place of publication
- Page numbers
- Volume number

- Issue number - URL (website or web page address) - DOI (link for journal articles)
- Title of conference proceedings - Report number - Book or conference editor
- Book or conference title - Journal title - Date of access (for online material)

The more references you have to write, the more familiar you will be with what you need to know. If you are unsure, check our guides, ask us, or check with your lecturers.

7. HOW DO I WRITE A REFERENCE LIST?

This is your list of all the sources that have been cited in the assignment. The list is inclusive showing books, journals, etc., listed in one list, not in separate lists according to source type.

- The list should be in alphabetical order by author/editor.
- Books, paper or electronic journal articles, etc., are written in a particular format that must be followed.
- Your reference list contains all the items you have cited or directly quoted from.
- When you have used more than one piece of work by the same author, in your reference list you should list the works in date order, beginning with the most recently published work.

10. HOW TO WRITE REFERENCES FOR YOUR REFERENCE LIST: HARVARD STYLE

Remember: Your lecturers consider accurate and consistent referencing to be an important part of your academic work. Always check your course guidelines so you know which style of referencing to use, and always use the help guides especially if you're using a new style.

The examples on the following pages are in two parts:

- the information you should collect about each piece of work you use; and
- how this information is presented when you write a full reference.

Book: print

Author/Editor (if it is an editor always put (ed.) after the name) (Year of publication) Title (this should be in italics) Series title and number (if part of a series) Edition (if not the first edition) Place of publication (if there is more than one place listed, use the first named) Publisher

Simons, N. E., Menzies, B. & Matthews, M. (2001) *A Short Course in Soil and Rock Slope Engineering*. London, Thomas Telford Publishing.

Journal article: print

Author (Year of publication) Title of journal article Title of journal (this should be in italics)
Volume number Issue number Page numbers of the article (do not use 'p'. before the page numbers)

Chhibber, P. K. & Majumdar, S. K. (1999) Foreign ownership and profitability: Property rights, control, and the performance of firms in Indian industry. *Journal of Law & Economics*, 42 (1), 209-238.

Journal article: online/electronic

If an electronic journal article has a DOI (digital object identifier), you can use this instead of the URL. The DOI is a permanent identifier provided by publishers so that the article can always be found online. Your lecturer may ask you to include the DOI, not a direct URL, in your written references.

To find the DOI, when you read an article online, check the article details as you will usually find the DOI at the start of the article. For more help, contact your librarian.

If you read the article in a full-text database service, such as Factiva or EBSCO, and do not have a DOI or direct URL to the article you should use the database URL.

Author (Year of publication) Title of journal article Title of journal (this should be in italics)
[Online] Volume number Issue number Page numbers of the article (do not use 'p'. before the page numbers) Available from: URL or DOI [Date of access]

Arrami, M. & Garner, H. (2008) A tale of two citations. *Nature*. [Online] 451(7177), 397-399. Available from: <http://www.nature.com/nature/journal/v451/n7177/full/451397a.html> [Accessed 20th January 2008].

or

Wang, F., Maidment, G., Missenden, J. & Tozer, R. (2007) The novel use of phase change materials in refrigeration plant. Part 1: Experimental investigation. *Applied Thermal Engineering*. [Online] 27 (17-18), 2893-2901. Available from: doi:10.1016/j.applthermaleng.2005.06.011 [Accessed 15th July 2008].

or

Read, B. (2008) Anti-cheating crusader vexes some professors. *Chronicle of Higher Education*. [Online] 54 (25). Available from: <http://global.factiva.com/> [Accessed 18th June 2009].

Note: articles published online may not have page numbers.

THE DIAGNOSIS OF PLANT DISEASE

Diagnosis of a plant disease is dependent mainly on observations of the symptoms of the disease and the presence of a pathogenic agent in or on diseased tissues.

There are three main symptom types that are characteristic of plant disease.

- a. Necrosis or death of infected tissue — e.g. leaf spot of Banana caused by *Mycosphaerella musicola*.
- b. Hyperplasia and hypertrophy — increase in cell number and size respectively, resulting in galls, tumours or witches' broom, e.g. witches' broom disease of cocoa caused by *Crinipellis perniciosus*.
- c. Hypoplasia and hypotrophy — reduced growth and stunting, characteristic of plants attacked by plant parasitic nematodes.

The major agents producing these symptom types are either fungi, bacteria, viruses, mycoplasma, nematodes, insects or so-called physiological disorders or non-parasitic disease agents. The latter include adverse climatic conditions, lack of a particular nutrient, phytotoxic atmospheric pollutants, e.g. sulphur dioxide, fluorine, ozone, etc.

The determination of the causal agent of a disease is usually the first step in attempts to control the disease. Where the disease is common and experience in diagnosis is available, there is no need to isolate the pathogen; however, in some cases the information is not available and isolation may be necessary.

It is sometimes felt that diagnosis is a simple matter of looking at the plant and immediately the disease is known and complete recommendations can be given for control. But this is not like the medical doctor/patient situation. Plants cannot talk, and consequently, the approach to diagnosis must be cautious. A tomato

plant which wilts in the field on a sunny day may be affected by one or more of the following:

- a. Bacterial wilt caused by *Pseudomonas solanacearum*.
- b. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*.
- c. Nematode attack – e.g. *Meloidogyne* spp.
- d. Insect damage to roots.
- e. Lack of water.
- f. Toxic conditions in the soil.
- g. Poor root development owing to poor soil physical conditions.

Consequently a careful evaluation of the total situation is necessary before a correct diagnosis is made.

Steps in diagnosing a disease

- a. Examine the plant, carefully noting the following:
 - 1) parts of the plant attacked – young vs. old leaves, etc.
 - 2) the kinds of symptoms produced; for example, if there are leaf spots, are they angular? Coalescing? Surrounded by a halo? Do they have a distinct margin?
 - 3) evidence of the pathogen; sometimes, conidiophores or conidia of the pathogen are obvious. They can be scraped from lesions and examined under the microscope.
 - 4) field conditions and cultural practices.
- b. The total disease syndrome, is compared with the description of the diseases in a recognized text.
- c. If there is no text or description of the disease, it will be necessary either to obtain the services of a competent plant pathologist or attempt to isolate the pathogen.

Precautions in diagnosis

- a. Never overlook the possibility of several diseases occurring on the same plant or part of the plant.
- b. There are several cases of insect damage that can be mistaken for disease caused by fungi, bacteria or viruses.

- c. The cultural conditions under which a crop is grown must be carefully considered.

COLLECTING SAMPLES

Very often, it is not possible to diagnose the disease, and samples must be sent to competent authorities. The following hints in sampling should be useful:

- a. Collect good quality specimens.
- b. Collect all parts of the plant which show evidence of disease.
- c. Wrap specimens in moist paper and enclose in a plastic bag.
- d. Where it is necessary to preserve specimens for long-term storage, place plant parts in Formalin-acetic acid-alcohol (FAA). This is made up of 50% ethanol (100 ml), Formaldehyde (10 ml) and acetic acid (10 ml).
- e. Photographs of the diseased specimen may also be helpful in diagnosis.

2

THE MICROSCOPE

Because most organisms that cause plant disease are too small to be seen with the naked eye, a microscope is an important tool in Plant Pathology. From the standpoint of construction and operational details, there are many different kinds of microscopes; however, the principles underlying their operation are the same. The microscope basically consists of two systems:

- a. an optical system (for magnification).
- b. an illumination system (for rendering the specimen properly visible).

The optical system

Magnification in the compound microscope is obtained by a series of two lens systems. The lens nearest the specimen is called the **objective**, and that nearest the eye is called the **ocular** or **eyepiece**. The total magnification is the product of the ocular and objective magnifications. The objective lens is made of a combination of convex and concave lenses. The lenses are usually made of different kinds of glass to correct for various chromatic and spherical aberrations. Microscopes are usually fitted with three objectives: (1) the low power objective (16 mm, magnification $\times 10$), (2) the high-dry objective (4 mm, $\times 40$) and (3) the oil immersion objective (1.8 mm, $\times 95$). The millimetre reading designates the focal length of the objective. The shorter the focal length, the shorter is the distance between the specimen and the objective. Some microscopes also carry an extra low power objective ($\times 4$). The objectives are held and rotated into place by a revolving nosepiece. Two adjustment wheels focus the lens system on the specimen. The **coarse adjustment** moves the body tube (or the stage on some models) over a greater vertical distance and brings the specimen into approximate focus, while the **fine adjustment** moves the body tube less for precise focusing.

Some microscopes are fitted with a condenser, a lens system which gathers the light and focuses it on the specimen. The condenser is usually equipped with an iris diaphragm which regulates the amount of light entering the condenser. There may be provision for the use of filters.

Magnification of the light microscope

The smallest diameter that can be seen with the standard light microscope is about 0.2μ ($1 \mu = .001 \text{ mm}$). Bacteria, fungi and nematodes are all larger than 0.2μ , but viruses and mycoplasma are smaller than 0.2μ and can only be "seen" with the electron microscope.

The illumination system

The illumination system may consist of a simple mirror or a built-in light source.

CALIBRATION OF THE MICROSCOPE

For measuring a microscopic object or specimen, a calibrated microscope is necessary. In order to calibrate the microscope, an ocular micrometer and a stage micrometer are needed.

Procedure:

- a. Unscrew the upper lens eyepiece and place the ocular micrometer (engraved side up) on the metal shelf (i.e. the ocular diaphragm) on the inside of the eyepiece.
- b. Place the stage micrometer (right side up) on the stage of the microscope under an objective. Locate the scale on the stage micrometer under the objective.
- c. Superimpose the scale on the ocular micrometer onto the scale of the stage micrometer, such that the graduations or divisions on one scale are parallel with those on the other.
- d. At one end of the scales, select 2 lines that coincide. Then look for another line on the ocular micrometer that coincides with one on the stage micrometer.
- e. Determine the number of divisions on the ocular micrometer and those on the stage micrometer between the lines that coincide.

- f. If the scale on the stage micrometer is 1 division = .01 mm, and 45 ocular divisions equal 35 objective (or stage) divisions, then 1 ocular division equals $\frac{35}{45} \times .01 = 0.0077$ mm or 7.7μ .
- g. The values obtained are valid only so long as the same ocular micrometer in the same eyepiece is used on the same microscope and at the same tube length.
- h. The procedure must be repeated for each objective or when any of the factors indicated in (g) above is changed.

USE OF THE MICROSCOPE

- a. Place the slide on the stage, specimen side up, and centre the section to be examined as accurately as possible over the hole in the centre of the stage.
- b. Adjust the mirror so that the maximum amount of light reaches the specimen.
- c. With the low-power objective in position, lower the body-tube until the objective is about 0.5 cm from the slide.
- d. Look through the eyepiece and slowly raise the objective until the specimen is in approximate focus. N.B. Never focus downward while looking through the eyepiece; that may be the end of your specimen.
- e. After examining the specimen at low-power, shift to high-dry objective by rotating the nose-piece until the objective clicks into place.
- f. Look through the eyepiece and slowly raise the tube, using the coarse adjustment, until the specimen comes into focus. Bring the image into accurate focus with the fine adjustment.
- g. Care must be taken in using the oil immersion lens. Raise the body tube and rotate the nose-piece until the oil immersion objective clicks into position. Now place a drop of immersion oil on the portion of the slide directly under the objective. Watching the objective from the side, carefully lower it into the oil. Do not allow the objective to touch the slide. Look through ocular and slowly focus upward, using the fine adjustment. The image should come into view.

The microscope

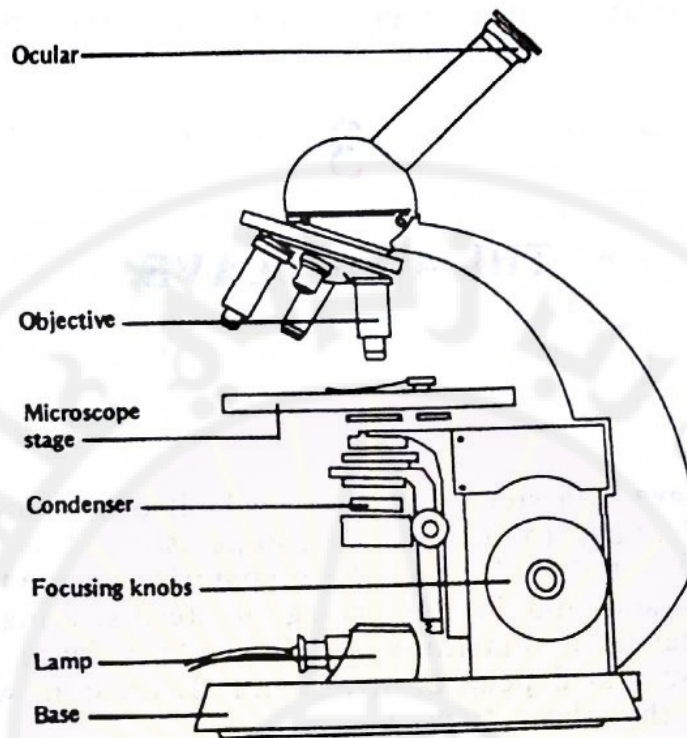


Fig. 1. Diagrammatic representation of a typical compound light microscope.

Care of the microscope

- a. Never touch microscope lenses with the fingers.
- b. Clean the lenses by gently wiping with lens tissue only, or with lens tissue dipped in xylene or diethylether.
- c. Keep the stage of the microscope clean and dry.
- d. Do not tilt the microscope when working with oil immersion lenses. The oil may drip onto the stage condenser and harden, thus destroying the condenser.
- e. Never force the microscope — all components should work freely.
- f. Never allow the objective to touch the slide or coverslip.
- g. Never lower the body-tube with the coarse adjustment while looking through the microscope.
- h. Never exchange lenses of different microscopes.
- i. Store microscope in microscope box when not in use.
- j. Before using any microscope, always take a few minutes to read the instruction manual. The few minutes spent at this time may make the difference between frustration and success.

THE AUTOCLAVE

The autoclave is a sterilizing device which uses moist heat in a pressurized chamber to destroy microorganisms. It is an essential tool used in Plant Pathology in the preparation of media, sterilization of equipment and in the drying of sterilized materials. The pressure cooker is a miniature autoclave. Autoclaves are of two types: 1) those that depend on live steam; 2) those in which steam is generated within the autoclave.

The autoclave is effective in killing microorganisms because: 1) the material to be sterilized is first moistened; 2) the steam is at a temperature of about 121°C ; 3) the pressure is about 15-20 psi.

The moist heat is believed to cause irreversible coagulation of proteins in the living cells.

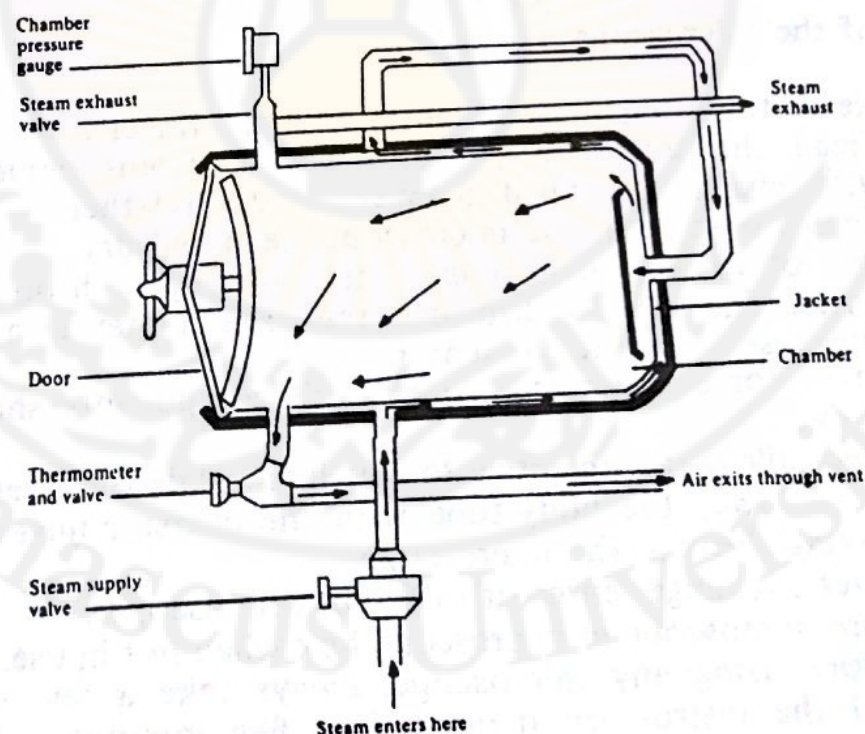


Fig. 2. Diagrammatic representation of a typical steam autoclave.

THE OPERATION OF AN AUTOCLAVE IN WHICH STEAM IS GENERATED IN THE UNIT

- a. Check to ensure that sufficient water is present in the autoclave.
- b. Load the autoclave and close lid securely.
- c. Open the steam valve and switch on electricity supply.
- d. Let steam issue from the valve for about 5 minutes, then close the valve.
- e. Allow the autoclave to operate for the required time.
- f. Turn off electric supply.
- g. Let pressure drop to zero, then open steam valve.
- h. Wait 5 minutes, then open lid.

THE PREPARATION OF MEDIA FOR FUNGAL AND BACTERIAL GROWTH

CONSTITUENTS OF A MEDIUM

A variety of solid and liquid media is used for the growth of fungal and bacterial pathogens. A suitable medium for growth of these pathogens should include available sources of carbon, nitrogen, inorganic salts and, depending on the organism, even vitamins or other growth substances. These may be supplied in the form of natural products such as sterilized plant parts (leaves, shoots, roots, seeds etc.), infusions or extracts of these materials, or chemically defined synthetic mixtures of known compounds.

The plant pathogenic nematodes and viruses and certain fungi such as some rust fungi, powdery fungi, and some of the downy mildew fungi, have not yet been grown successfully on a nutrient medium. These pathogens are called obligate parasites.

Liquid media usually have the same composition as their solid counterparts, except that agar is included in solid media. Agar is a complex polysaccharide which is usually obtained from a sea alga. Agar dissolves in water at 100°C and solidifies at 40°C to form a relatively clear gel. Agar is usually added to media at 1.5 to 2 percent, i.e. 15-20 gm per litre of medium.

Most of the media used routinely in plant disease investigations are easily prepared. In general, the procedure is as follows:

Add the ingredients, in the amounts indicated in the formula or recipe, to a beaker or Erlenmeyer flask that will hold 1 – 1 1/2 or 2 times the final volume of medium. Measure the required volume of water. Begin adding water to the ingredients, a little at a time, to prevent the formation of lumps, stirring constantly. When the ingredients are thoroughly moistened, the rest of the water can be added. Aluminium foil may be used to cap the flask or beaker. Heat the flask and its contents to dissolve the ingredients. When agar is a component of the medium, the mixture must be heated to the boiling point, to dissolve the agar. When antibiotic and other heat labile sub-

stances are being used in media, they must be sterilized by filtration and added to the cooled, autoclaved medium.

pH OF THE MEDIUM

An important factor to be considered in media preparation is the pH of the medium. Fungi usually grow better under acid conditions (about pH 5.5-5.8), while the growth of bacteria is usually favored by media approximately neutral in reaction (about pH 6.8-7.0). The reaction of the medium can be determined readily by means of pH indicator papers, indicator dyes, or a pH meter. The reaction of the medium can be adjusted to the desired level by adding 1N NaOH or HCl to the hot solution. The pH of a medium can also be controlled by making up the medium in a buffer rather than water.

DISPENSING THE MEDIUM

While it is still hot (in the case of an agar medium), the mixture is dispensed into appropriate containers for sterilization. If the medium is to be used for propagation of a fungal or a bacterial pathogen, it is usually dispensed into test tubes. Six-inch tubes with a 3/4" diameter are the most common. If the medium is to be stored or used in petri plates, it can be dispensed into 300-ml Erlenmeyer flasks or 8-oz screw-top medicine bottles. Dispensing the medium can be facilitated with the use of a 500-1000 ml capacity funnel, fitted with a short piece of rubber tubing that ends in three inches of glass tubing. The funnel is supported by a ring stand, and delivery of the medium to the tubes or bottles is controlled by a pinch-cock attached to the rubber tubing. About 12 ml of medium is dispensed into each tube. The flasks should not contain more than 250 ml of medium; they can then be plugged with cotton or capped with aluminum foil. If medicine bottles are used, they should not be filled beyond the shoulder of the bottle; the caps should be in position during sterilization, but fitted loosely to allow for expansion of the medium and air within the bottle during sterilization. The caps are tightened prior to removing the bottles from the sterilizer.

STERILIZATION OF THE MEDIUM

A medium must be sterilized the same day it is prepared in order to prevent microbial growth. Sterilization of the medium should be done in a steam autoclave or in a pressure cooker. Tubes should be

arranged loosely in wire baskets or other containers, and flasks or medicine bottles should be located in the sterilizer so as to permit free movement of flowing steam around them. Sterilization is completed in 20 minutes at 15 lbs pressure and a temperature of 121°C. After the required time has elapsed, the pressure should be allowed to fall gradually to zero before the medium is removed from the sterilizer. If the pressure is released too quickly, the medium may boil over from the containers, wetting the cotton plugs and causing the medium to be lost from the containers. Jarring or swirling the tubes or flasks while they are being removed from the sterilizer may also cause the medium to boil over and result in painful burns to the investigator. It is important to handle them with great care during this operation until they have cooled.

PREPARING THE MEDIUM FOR SLANT CULTURES AND PLATE CULTURES

Tubes containing an agar medium are arranged in a slanted position to cool so that when the agar has solidified, a sloped surface will extend from about 1/2" to 3" – 3 1/2" from the bottom of the tube.

If the medium is to be added to petri plates, it should be allowed to cool to about 50°C (at this temperature the flask can be held against the face or forearm). The sterile petri plates are stacked in groups of 5 or 6. The melted agar is then added to each dish in turn, beginning with the lowest dish by raising its lid just enough to pour the agar from the flask into the dish. Add enough agar to provide a medium depth of about 5 mm, or about 15-20 ml for standard petri plates.

SOME COMMONLY USED MEDIA

a. Potato dextrose agar – PDA

Ingredients:	Distilled or tap water	1000 ml
	Sliced potatoes	200 g
	Agar	17 g
	Dextrose	20 g

Procedure: Cook potatoes in 500 ml of water for 20-30 minutes over a low fire. Strain the potato broth through 2 or

3 layers of cheesecloth to remove potato debris. Melt the agar in 500 ml of water in an autoclave or pressure cooker and add 20 g of dextrose to the melted agar. Combine the hot potato broth and melted agar solutions and bring to a volume of 1000 ml. Mix thoroughly and dispense the medium into test tubes or flasks plugged with cotton and sterilized.

b. Nutrient agar (NA)

Ingredients:	Distilled or tap water	1000 ml
	Beef extract	3 g
	Peptone	5 g
	Agar	15 g

Procedure: Heat the beef extract, peptone, and agar in water until they dissolve. Dispense the medium into tubes, flasks or bottles which are plugged with cotton or suitably capped (e.g. with aluminium foil) and sterilized. The final reaction of the medium will be about pH 6.8. Nutrient broth is prepared in the same manner, except that agar is omitted from the preparation.

c. Water agar (WA)

Ingredients:	Distilled water	1000 ml
	Agar	15–20 g

Procedure: Dissolve agar in the water by heating. If desired, adjust the pH with colorimetric pH indicators or a pH meter and the addition of 1N HCl or NaOH. Dispense the medium into tubes, flasks or bottles, plugged with cotton or capped and sterilized.

d. Lima bean agar

Ingredients:	Distilled water	1000 ml
	Lima beans	200 g
	Agar	15 g

Procedure: Blend frozen lima beans and water in a kitchen blender until the mixture is smooth. Add agar to the mixture, then heat to dissolve the agar. Dispense the medium into tubes etc., and autoclave.

e. Oatmeal agar

Ingredients:	Distilled water	1000 ml
	Oatmeal	100 g
	Agar	15 g

Procedure: Stir Oatmeal into water at about 70°C, and maintain at 60°C for about 1 hour. Restore the volume, add agar, and heat to dissolve. Dispense the medium into tubes etc., and sterilize.

SELECTIVE MEDIA

The addition of certain chemicals to a basal medium can repress the growth of certain microorganisms while permitting the growth of the organism required. This repressive selection is usually used for isolating plant pathogens from soil or from infected tissue. The addition of antibiotics to the medium removes many bacteria, as required for the isolation of fungal pathogens. Generally, the presence of 100 ppm of streptomycin will inhibit growth of most bacteria. Altering the pH can also be effectively used to eliminate bacteria during isolation. Because bacteria grow best at pH 6.8 to 7.0 and most fungi at 5.0 to 5.8, a medium with a low pH will thus inhibit bacterial growth, while favoring fungal growth. One or two drops of a 25% solution of lactic acid are usually added to 10 ml of melted agar to inhibit bacterial growth when isolating fungi.

CLASSIFICATION OF MEDIA

Media for microbial growth may be described as **synthetic**, **semisynthetic** or **natural**. Synthetic media are those whose chemical composition is known precisely. Semisynthetic media are those in which a few of the ingredients are known precisely, while natural media are derived from complex plant or animal products and their precise composition is unknown, e.g. oatmeal agar. The composition of oatmeal agar will depend on the composition of the oat used in the medium which, in turn, will depend on the variety of oats, the age of the grain and several other factors.

STERILIZATION OF EQUIPMENT

When sterile glassware such as pipettes, petri plates or flasks are required for an experiment, the glassware should be washed in a detergent, thoroughly rinsed with tap water, then with distilled

water, and allowed to dry. Flasks need only to be capped with cotton or aluminium foil. Pipettes should be sorted to size and then wrapped in newspaper or wrapping paper; petri plates can be placed in lots of six or twelve in paper bags of appropriate size or wrapped in newspaper or aluminium foil. The paper wrapping should be secured with sticky paper tape prior to heating the glassware. Sterilization is accomplished by heating the glassware in an oven at 180°C or 356°F for a minimum of 2 hours. To prevent damage, containers or equipment containing rubber or plastic parts should be sterilized in an autoclave for 20 minutes.

5

DETECTION OF FUNGAL PATHOGENS IN INFECTED PLANT TISSUES

In some cases, the presence of a fungal pathogen in infected tissues is revealed by the presence of the reproductive or vegetative structures of the fungus on the surface of these tissues, e.g. spores of powdery mildew on cucumber leaves. In other cases, however, some magnification of the specimen is necessary. This may be done either with a simple hand lens (15-20 x magnification) or with a microscope (either stereoscopic or compound).

Many fungi produce their reproductive units called spores or conidia on stalk-like structures that emerge from the plant tissues. The spores are variously shaped, sized and colored. Examples include: the large, dark, cigar-shaped spores of *Helminthosporium* that cause lesions on cereal and grass leaves; the small hyaline oval or round spores of *Botrytis* that are produced in grape-like clusters on flowers during wet periods; the hyaline, barrel-shaped spores of the mildew fungus *Erysiphe* that are produced in chains on the leaves of cereals, lettuce and certain other plants. Numerous fungi produce their spores in tiny flask-shaped structures called pycnidia. When moist conditions prevail, the spores ooze out in a mucilaginous matrix and appear as glistening droplets surmounting the tiny pycnidium.

Sometimes the fungal pathogen is not apparent when diseased tissue is examined microscopically. In such instances, if the plant material is washed in a detergent solution, rinsed well in water and then placed in a closed container (petri dish, test tube, jar, etc.) containing moist filter paper or paper towels, the pathogen usually will grow out of the diseased tissues and produce its reproductive structures on the surface.

Mycelium in plant tissues is readily differentiated by using the differential stain, cotton blue, in lactophenol. Lactophenol solution is prepared from 20 grams of phenol (warmed until melted), 20 ml lactic acid, 30 ml glycerine and 20 ml distilled water. To one-half of the lactophenol solution is added 2.5 ml of a 1.0% cotton blue (aniline blue) solution in distilled water. Thinly sliced sections or

bits of diseased tissue are placed on a slide, and a few drops of the stain are added. The slide is warmed in a flame until the solution fumes. The plant material is then rinsed in the clear lactophenol solution, transferred to 1-2 drops of lactophenol solution on a new microscope slide, covered with a coverslip and observed with the microscope. The fungal mycelium is stained blue while the plant tissue remains unstained. Care should be taken not to allow the microscope objectives to contact the lactophenol solution, thus causing damage to the objectives. Caution should be exercised in handling the phenol since it may cause severe burns.

ISOLATING A FUNGAL PATHOGEN

Bacteria and fungi are ubiquitously present as contaminants on plant tissue. Since the goal is to isolate the pathogenic fungus from the diseased tissues and to grow it as a pure culture, it is important to exclude bacteria and fungi that are present as contaminants on the surface of the working area, on equipment to be used, and on the surface of the diseased tissue.

The surface of the working area may simply be wiped with a cloth moistened with water, or with a disinfectant such as mercuric chloride (1:1000) or sodium hypochlorite solution.

Prior to using a scalpel, dissecting needle, forceps etc., the instrument should be dipped in 70% ethanol and then passed through a flame to eliminate contaminants from that portion of the instrument that will be used in handling the diseased tissue. The instrument should be air cooled for a minute before use.

Contaminants on the surface of woody plant parts such as twigs and roots are easily destroyed by dipping the plant part in 70% ethanol, passing the material through a flame and allowing the alcohol to burn off. This treatment is usually too drastic for succulent tissues, such as leaves, shoots, flower parts or seedling tissues. For these, household bleach is used, often in combination with a wetting agent if the surface of the plant tissue is difficult to wet.

The active ingredient in preparations of household bleach is sodium hypochlorite at a concentration of about 5.25% by weight. The bleach solution is diluted with water (10:90) to give a 10% (volume/volume) solution of disinfectant. It is sometimes necessary to ensure complete surface disinfection by rinsing plant materials that are difficult to wet in 70% ethanol or in a solution of a wetting agent (Agral, 1-2 drops/1000 cc of water) or a 2% solution of Decon 90 prior to immersing them in the disinfectant. If the diseased area is small, as in leaf-spots or stem lesions, the diseased tissue and a small amount of adjacent tissue is cut out, rinsed in wetting agent and immersed in the disinfectant. If the affected area involves a large portion of the leaf, shoot, etc., as in some blights,

a portion of plant tissue, which preferably includes both diseased tissue and healthy tissue adjacent to the margin of the diseased area, is removed, rinsed in a wetting agent and immersed in the disinfectant. About 1 cm² of plant tissue can be handled conveniently in this manner and provides enough tissue to prevent the disinfectant from penetrating the tissues and killing the pathogen. The time that the tissue is left in the disinfectant varies according to the type of tissue involved and may range from 1-2 minutes for thin, very succulent tissue to 10-15 minutes for hard seeds.

Additional disinfectants are:

- a. 95% alcohol — dip for 3 seconds.
- b. 50% hydrogen peroxide — dip for 15 seconds to 5 minutes.
- c. 0.4% Formalin — dip for 15 seconds to 5 minutes.

PLATING THE INFECTED TISSUE

The disinfested tissue may be blotted dry between paper towels and then cut into small pieces 1-2 mm² in size which are transferred to the surface of an agar medium in petri plates. The lid of the petri plate should be raised only enough to allow the bits of tissue to be placed readily on the agar. Four or five pieces of tissue should be spaced approximately equidistant on the plate. The bark of woody tissue is removed using sterile forceps, and small chips or bits of tissue are transferred to the medium in petri plates, as described for more succulent tissue.

The plates are usually kept at a temperature of 25-30°C for a few days to allow the pathogen to grow out of the tissue and develop on the agar medium. When the type of fungal growth from the tissues is consistent for several pieces of tissue, it can be assumed that the pathogen has been isolated.

ESTABLISHING PURE CULTURES

Some of the fungal growth from each of several colonies should be transferred to individual tubes of the same agar medium to provide pure cultures of the pathogen. This is most readily done by means of a transfer needle. The needle is heated in a flame until it glows red, and then cooled by touching it to a portion of the agar medium that is free of microbial growth. A tiny portion of the medium and fungal growth is then removed from the fungal colony and transferred to the midpoint of the sloped surface of

the medium in a tube. The pathogen usually can be maintained in pure culture for long periods by storing it at about 5°C and subsequently transferring it to tubes of fresh medium in a similar manner at intervals of 2-3 months.

DETERMINING THE CHARACTERISTICS OF THE ISOLATED FUNGUS

Characteristics of the growth of a fungal colony growing on an agar medium in a petri dish can be observed through the microscope by placing the uncovered petri dish on the stage of the microscope and gently placing a cover slip over the advancing margin of the fungal colony. Characteristics of mycelium and spores can be observed by using a dissecting needle or transfer needle to transfer bits of the fungal growth from a culture to a small drop of water on a microscope slide, placing a cover slip gently on the drop and viewing the material through the microscope.

The slide culture technique is especially useful for determining the mode of sporulation of a fungal pathogen. A petri dish is fitted with two pieces of 9 cm diameter filter paper, a bent glass rod, a microscope slide and a 22 x 30 mm cover slip. The petri dish and contents are sterilized at 15 lbs. pressure and a temperature of 121°C for 15-20 minutes. Sterile water agar or PDA (2-3% agar) in petri plates is cut with a sterile scalpel into squares smaller than the cover slip. A block of agar is then transferred to the sterile slide in the petri plate, and the cover slip is placed on it with sterile forceps. Bits of mycelium and/or spores are transferred from a culture of the fungus to the mid-point of each of the four sides of the agar block. The filter paper in the dish is moistened with an aqueous solution of 5% glycerin to maintain the humidity of the dish. The plates are kept at 25-30°C for a few days to allow the fungus to grow and sporulate. The slide cultures are removed to the microscope stage for observation. For many fungi it is possible to determine the characteristics of the mycelium, the manner in which the spores are produced and characteristics of the spores.

Fungal structures may be more clearly apparent if they are stained with 1% solution of acid fuchsin or cotton blue in lactophenol. One or two drops of the stain are added to a microscope slide, and the fungal mycelium is transferred to the drop, which is then covered with a cover slip. If the fungal material is mounted in water, stain can be added to the mount by placing a drop of stain on the slide at the edge of the cover slip and then drawing the drop of stain under the cover slip by bringing a piece of filter paper into contact with the water at the opposite edge of the cover slip.

OBSERVING THE DEVELOPMENT OF A FUNGUS ON LEAF SURFACES

The germination of fungal spores on the leaf surface, and their penetration into leaf tissues, can be followed readily by what is called the whole-mount technique. At intervals (e.g. 6, 18, 32 and 48 hours) after a plant is inoculated with spores of a fungal pathogen, leaves are collected and placed in Carnoy's solution (2:1 mixture of glacial acetic acid and absolute ethyl alcohol) for 24 hours, then transferred to lactophenol solution for 24 hours, stained in 0.1% acid fuchsin in lactophenol solution for 8-10 hours, rinsed in lactophenol solution and finally mounted in lactophenol solution.

DETECTION OF BACTERIAL PATHOGENS IN INFECTED TISSUES

The presence of plant pathogenic bacteria in infected plant tissue can be demonstrated, and their size and shape determined, by a technique that involves negative or background staining. A drop of 2% solution of Congo red stain in distilled water is placed on a clean microscope slide. A sterile scalpel is used to remove a small piece (1-2 sq. mm) of diseased tissue from the margin of healthy and diseased tissue, which is placed in the drop of Congo red solution and crushed with the scalpel. After 1-2 minutes the bits of tissue are scraped from the slide, and the remaining stain solution is spread in a thin film over the slide. The stain is allowed to dry without heating and then flooded with 2-3 drops of acid alcohol (3 drops concentrated HCl in 30 ml 95% ethanol). The acid alcohol turns the stain blue. The alcohol is allowed to evaporate. A drop of immersion oil is added directly to the stained portion of the slide, and the slide is examined directly with the oil immersion lens of the microscope. If an oil immersion lens is not available, a drop of mineral oil covered with a cover slip can be used with the high power lens. The bacteria appear as unstained (white) short, rod-shaped cells against a blue background. If the stain is not spread thinly enough over the slide, the slide will appear black or very dark blue, and the bacteria will not be visible. If too much plant tissue is used to prepare the slide, the stain may be precipitated and fail to give the desired result.

ISOLATION OF BACTERIAL PATHOGENS

A simple procedure for isolating bacterial pathogens in pure culture involves preparation of a dilution series.

- a. Arrange 6 sterile petri plates in a row and label them 1 through 6.
- b. Place 4 drops of sterile water separately and equidistantly in the first petri plate.

- c. Place a single drop of water in each of the remaining petri plates.
- d. Use a sterile scalpel to remove a small piece of diseased tissue from the affected plant and place the tissue in one of the drops on the first petri plate.
- e. Use the scalpel to crush the diseased tissue in the drop of water.
- f. After 5-10 minutes (to allow the bacteria to separate from the plant tissue), use a transfer loop that has been flamed and cooled to transfer a loopful of water and bacteria from the first to the second drop in the same plate. The second drop is mixed well with the transfer loop, and **without flaming the loop**, a loopful is transferred from the second drop to the third drop in the plate. This operation is repeated successively from drop to drop and plate to plate and results in a progressive dilution of the number of bacteria in the original suspension.
- g. Add cooled, melted nutrient agar to each plate and swirl the plate to distribute the bacteria uniformly throughout the agar.
- h. Store the plates at room temperature (25-30°C).
- i. In plates prepared at the higher dilutions, the bacteria will grow as distinct colonies; these presumably develop from single bacterial cells.
- j. When bacterial colonies appear in the plates, transfer cells from a single bacterial colony to a slanted tube of nutrient agar or nutrient broth.
- k. After 24 to 48 hours of growth, add sterilized distilled water to the nutrient agar slant and wash the cells into suspension with a flamed inoculating needle.
- l. Streak the suspension from the nutrient agar slant or the nutrient broth onto 2-3 agar plates, and observe the colonies after incubating the plates at room temperature for 24-48 hours. If all the colonies on the plates are identical in appearance and cultural and morphological characteristics, a pure culture has been obtained. Pathogenicity and other tests are then necessary to confirm the identity of the isolated bacterium.

OBSERVATION OF LIVING BACTERIA

Living bacteria are somewhat difficult to observe because the cells are small and contrast only slightly with their environment. However, they can be observed, and their motility can be studied, by using a hanging drop mount.

The procedure is as follows:

- a. Apply a smear of Vaseline around the depression of a depression slide.
- b. Using the inoculating loop, aseptically transfer one small drop of the culture to the centre of a clean square cover slip.
- c. Invert the depression slide and centre it over the drop of culture.
- d. Press down on the edge of the cover slip so that the Vaseline makes a firm seal.
- e. Quickly and carefully turn the slide over so that the drop is suspended in the depression of the slide. The drop must not touch the bottom of the depression.
- f. Examine the bacteria in the drop by first focusing on the edge of the drop with low power objective. Then, switch to high dry and re-focus on the drop. Bacteria should now be visible moving in the drop.

THE GRAM STAIN FOR BACTERIA

The Gram Stain is a differential staining technique which divides bacteria into two groups. The first of these groups is stained purple by the Gram Stain and is called Gram Positive, while the second group is stained pink and called Gram Negative. The technique is very useful in plant pathology because almost all genera of phytopathogenic bacteria except *Corynebacterium* and *Nocardia* are Gram Negative.

Four solutions are used in the Gram Stain:

- a. Crystal violet
- b. Gram's iodine
- c. 95% alcohol
- d. Safranin

The method of preparing these solutions is described at the end of the procedure.

Procedure:

- a. Place a loopful of the bacterial culture on a clean glass slide.
- b. Spread the drop to form a thin film.
- c. Allow the film to dry in air.
- d. Pass the slide rapidly through a Bunsen flame about three times (film side up). This fixes the bacteria to the slide.
- e. Stain with crystal violet for 30 seconds, either by adding drops of the stain to the smear, or by dipping the slide into the stain.
- f. Wash with tap water until the stain ceases to flow from the slide.
- g. Cover the film with Gram's iodine and allow to act for 30 seconds.
- h. Rinse in tap water.
- i. Rinse with 95% alcohol for 10 to 20 seconds (i.e. until drippings from slide are colorless).
- j. Rinse with tap water.
- k. Stain with safranin for 10 seconds.
- l. Rinse with tap water and blot dry.
- m. Examine under the oil immersion lens of the microscope.
- n. Determine the Gram reaction by noting the color of the cell wall.

Reagents for Gram Stain

Crystal Violet. One volume of a saturated alcoholic solution of crystal violet in four volumes of 1% aqueous ammonium oxalate. Allow ammonium oxalate solution to stand overnight or heat gently until in solution. Then mix with crystal violet solution.

Gram's iodine. Iodine crystals 1.0 gm, distilled H_2O 300 ml, potassium iodide 2.0 gm. Mix all the ingredients together.

Safranin. 100 ml of a saturated alcohol solution of safranin-O in one litre of distilled water.

DETERMINATION OF THE BACTERIAL POPULATION OF INFECTED TISSUES

The bacterial population of infected plant tissue can be determined by modifying the dilution plate-count technique. The tissue is measured (either by weight or by area), then surface disinfested

and ground in a mortar or blender with a small measured quantity of sterile distilled water. One millilitre of the suspension is transferred to 9 ml of sterile distilled water, and the resulting suspension is thoroughly mixed. One ml of this suspension is serially transferred to 9.0 ml of sterile distilled water to obtain a 10^9 dilution of the original suspension. Remove 1 ml of suspension from each dilution and place into a sterile petri plate. Add 15 ml agar medium to each plate and incubate the petri plates at room temperature. After 48 to 72 hours at room temperature, count the number of bacterial colonies on each plate. Determine the number of viable cells in the original sample by multiplying the number of colonies on the plate that have between 30 and 300 colonies, by the dilution factor. The results are given as the number of bacteria per gram or cm^2 of tissue.

Damascus University

KOCH'S POSTULATES

In determining the causal agent of a particular disease, it is not enough to have isolated a fungus or a bacterium from the infected plant. To demonstrate that an organism is the cause of a disease, the following procedure must be followed:

- a. Associate the organism with all cases of the disease.
- b. Isolate the organism and grow it in pure culture.
- c. Inoculate susceptible plants with the isolated organism and obtain identical symptoms to those previously observed.
- d. Re-isolate the organism and compare it with the original isolate. They should be identical.

These four steps have been called Koch's Postulates or Koch's Rules of Proof of Pathogenicity. They are named after Robert Koch, a pioneer in bacteriology, and when they are satisfied, the causal agent of the disease has been established.

INOCULATION TECHNIQUES

METHODS OF INOCULATING PLANTS WITH PHYTOPATHOGENIC BACTERIA

There are several techniques available for inoculating plants with phytopathogenic bacteria. The inoculation technique varies with the kind of disease.

Disease Type	Method of Inoculation
a. Soft rot, e.g. soft rot of carrots caused by <i>Erwinia carotovora</i> .	A bacterial suspension is dropped onto the cut surface of the tissue.
b. Wilt diseases, e.g. bacterial wilt of tomato caused by <i>Pseudomonas solanacearum</i> .	1) The roots of the plants can be cut back to 1-2 cm and then placed in a bacterial suspension for about 2 hrs. 2) The stem may be pricked with a needle dipped in bacteria suspension. Pricking is usually done in the area between the cotyledon and the first leaf.
c. Leaf spot diseases, e.g. Angular leaf spot of cucumber caused by <i>Pseudomonas lachrymans</i> .	1) A bacterial suspension may be sprayed onto the surface of the plant. 2) The surface of the leaf may be "painted" with a bacterial suspension. 3) A bacterial suspension may be injected into the tissue with a hypodermic needle.

After inoculation with bacteria, plants are usually placed in a moist chamber for 48 hours. This increases the chances of infection taking place, since plants are generally more susceptible to bacterial infection under conditions of high humidity.

METHODS OF INOCULATING PLANTS WITH PHYTOPATHOGENIC FUNGI

The method of inoculating plants with phytopathogenic fungi will vary depending on the nature of the disease and, more especially, on the process of infection. The inoculation techniques can be grouped into three main categories:

- a. **Leaf inoculation.** When the pathogen enters the plant through the leaves, leaf inoculation is used. A spore suspension or dry spores of the fungus may be rubbed, sprayed or brushed onto the surface of the leaves. Spore germination will take place, and infection follows. Moist conditions are usually maintained after inoculation.
- b. **Soil infestation.** Where the pathogen is soil-borne, spores, mycelia or sclerotia of the pathogen are mixed with sterilized soil, and healthy plants or seeds are then planted into the infested soil.
- c. **Seed infestation.** Seeds of the test plant may be mixed with spores of the fungus, and the seeds are planted into sterilized soil. Seed infestation is commonly used with smut diseases of cereals.

Plant Diseases

History: Plant loss to homeowners may result in frustration and minor monetary cost. However, on a global scale, plant diseases cause an estimated \$38 billion¹ in annual losses. History also provides some perspective on the impacts of plant disease.

One of the most notable historical impacts of plant disease was caused by late blight of potato. This disease was a major contributing factor in the Irish potato famine of 1845. During this time, approximately one million people perished from starvation; a million and a half more are believed to have left Ireland and immigrated to the United States. The late blight pathogen is still present in production systems today, but it is managed by resistant varieties, sanitation, and fungicides.

Near complete loss of the American chestnut was caused by a fungal disease that nearly wiped out forests in the eastern United States. The trees once grew to majestic heights within their native forest habitat, as well as in urban plantings. They provided high quality hardwood for building construction and nuts as a food source for people and wildlife. However, in the late 1800s, chestnut blight was accidentally introduced to the United States through imported Chinese chestnut trees. While Chinese chestnuts are tolerant to the blight, American chestnut trees are not, and in less than 40 years, approximately 30 million acres of chestnut trees died. Chestnut blight remains a problem, and researchers are still seeking options for management.

Disease management changed drastically when the French wine industry gave way to the first fungicide, Bordeaux mixture. France's grape production had long been devastated by powdery mildew and downy mildew. Then in 1882, the discovery of a copper sulfate and lime mixture helped manage these vineyard diseases. Bordeaux mixture is still used in a modified form to manage powdery mildew, downy mildew, and other fungal diseases on numerous types of plants.

These examples represent situations in which plant diseases have reached historical proportions. However, the amount of damage that plant diseases cause varies depending upon factors such as environment, host health and susceptibility, and pathogen biology. There are many options for managing disease development and spread. The effectiveness of management techniques begins with proper identification of the disease and/or causal organism.

Pathogens: A plant disease is any physiological or structural abnormality that is caused by a living organism. Organisms that cause disease are referred to as ‘pathogens,’ and affected plants are referred to as ‘hosts.’ Many organisms rely on other species for sources of nutrients or as a means of survival, but are not always harmful to the host. For example, saprophytic organisms obtain nutrients from dead organic material and are a vital part of many ecosystems.

Plant pathogens, on the other hand, utilize hosts for nutrients and/or reproduction at the hosts’ expense. Disease causing organisms include fungi, oomycetes (fungus-like organisms called water molds), bacteria, viruses, nematodes, phytoplasmas, and parasitic seed plants.

Once a pathogen infects a host, symptoms often develop. Symptoms are the outward changes in the physical appearance of plants. Symptoms take time to develop, and thus, disease development may be delayed for several days, weeks, months, or even years after initial infection occurs. Examples of symptoms include wilt, leaf spots, cankers, rots, and decline.

Physical evidence of pathogens (called ‘signs’) may also be observed on diseased tissue. Examples of signs include fungal fruiting bodies, bacterial ooze, nematode cysts, and fungal mycelia. Both symptoms and signs are utilized in making disease diagnoses.

Conditions for disease development: Disease development is dependent upon four conditions: a susceptible host plant, a favourable environment, adequate time, and a viable pathogen. All four of these factors must be present for disease to occur. If one of the conditions is not present then disease does not occur. By altering the susceptibility of host plants, the surrounding environment, and/or the viability of pathogens, the disease conditions can be broken and disease development prevented.

Host plant genetic makeup determines its susceptibility to disease. This susceptibility depends upon various physical and biochemical factors within the plant. A plant’s stature, growth habit, cuticle thickness (a protective outer layer on plant tissues), and shape of stomata (small openings that allow water, oxygen, and carbon dioxide in and out of plant tissues) are a few physical factors that influence disease development. Plants may also produce biochemical compounds that limit or prevent colonization or infection. Growth stage and ability to deter pathogens can also impact plant susceptibility to disease. For example, young leaves are often more susceptible to infection than mature leaves.

Environment plays an important role in disease development. Pathogens generally require specific environmental conditions for infection and spread. Most plant pathogens require high humidity and moderate temperatures. Other pathogens, such as bacteria and water molds, require surface water for spread. In some disease cycles, environmental conditions influence the development of symptoms. For example, extreme temperatures or drought can cause plant stress; this loss of vigor can increase host susceptibility to both infection and disease development. Other environmental factors affecting disease can include those resulting from planting and maintenance practices. For example, high density plantings can have higher relative humidity, while overhead watering increases leaf surface moisture needed by pathogens to infect plant leaves.

Pathogens: must be present and viable in order to infect plants and cause disease. Removal of infected plant parts and other remnants of pathogens makes them unavailable for infection. Fungicides also reduce amounts of inoculum (infective propagules) available for infection. Many pathogens, however, have developed specialized structures that ensure survival during adverse conditions. For example, several water molds and fungi are capable of surviving in soil for many years until conditions are favourable for infection. Pathogens may also survive winter temperatures and other harsh conditions in infected plant tissue. If a susceptible host and favourable environment are not available, some pathogens can assume a dormant state for many years.

Fungi: are the most abundant group of plant pathogens. There are thousands of fungi capable of causing plant diseases. These multi-cellular organisms are typically microscopic. The 'body' of a fungus is composed of filament-like threads called 'hyphae.' Masses of hyphae are called 'mycelia.' When large enough, these masses can be seen without the aid of a microscope. Powdery mildew is one example of a disease in which fungal mycelia is visible. Fungi reproduce via spores, which can be produced sexually or asexually. Spores vary in colour, shape, size, and function, and this variation can often be used by diagnosticians as a means to identify pathogens. Some fungi produce spores within sexual fruiting structures or asexual fruiting structures. While many of these fruiting structures are small and may require the aid of a microscope, some can be seen with the naked eye. Other types of fungi produce exposed or unprotected spores that are not enclosed in structures. These spore types are more sensitive to environmental conditions than enclosed spores.

Once a fungal spore makes contact with a plant surface, it germinates, much like a seed, as long as conditions are favourable for the pathogen. Hyphae emerge from spores and are capable of infecting plants via natural plant openings (such as stomata), through wounds, or by direct penetration of the plant epidermis. Upon infection, fungi utilize nutrients from their hosts. Many fungi produce additional spores as they grow, which aids in the spread of the pathogen.

Spores or mycelial fragments (these infective units are referred to as 'propagules') can be spread via wind, water, soil, insects, animals, and humans. Fungi spread not only from plant to plant, but also within a single plant. Fungi may spread through a single plant as a systemic infection by utilizing the vascular system or by splashing spores that result in multiple infection sites on multiple plant tissues. Common symptoms caused by fungi include leaf spots, wilts, blights, cankers, fruit rots, and dieback.

Oomycetes (Water molds): As the name implies, water is essential for survival, reproduction, infection, and spread of oomycetes (commonly called water molds). Water molds were once considered true fungi, but they are now classified as fungus-like organisms. Water molds and fungi are similar in appearance, as the 'body' is composed of hyphae that mass together to form mycelia. Downy mildew is an example of a disease with visible oomycete mycelia.

Common symptoms caused by water molds include leaf spots, blights, cankers, root rots, wilt, damping off, and dieback.

Bacteria: are microscopic organisms typically composed of single cells. About 200 types of bacteria are known to cause plant diseases. Due to their small size, a high-magnification microscope is required to observe bacteria. Occasionally, when a large number of cells are present, plants may be observed 'oozing' bacteria and other organic byproducts.

Bacteria are capable of rapid reproduction through a process known as binary fission. In this process, one cell divides to become two, then two divide to become four cells, and so on. Within a few hours one bacterial cell can become thousands, and under ideal conditions, populations can double in as little as 20 minutes.

Unlike fungi and water molds, bacteria are not able to penetrate plant tissue directly. They must infect via wounds or natural plant openings such as stomata. Free water is required for infection. Once inside plants, bacteria begin to reproduce immediately. Some types of

bacteria produce toxins or enzymes that degrade plant tissue, and the tissue is then utilized as a food source. Some bacteria can colonize vascular systems of plants, which results in restriction of water movement.

Bacteria spread by water/splashing rain, wind, or insects, and then move across plant tissues in surface water to reach wounds or natural openings. Some can survive for five or more years in soil, as well as in plant debris and cankers. Common symptoms caused by bacteria include leaf spots, blights, cankers, galls, wilt, dieback, and soft rots.

Viruses: are extremely small particles that require magnification of 100,000 times or more for observation. This can only be achieved with specialized equipment, such as an electron microscope. Diseases caused by viruses are often named for the first host plant for which symptoms were reported and/or the most common symptom. An example is tobacco mosaic virus; this virus was first reported as a mosaic symptom on tobacco. However, this disease is known to infect more than 100 plant species, including many vegetable and ornamental plants.

Once viruses enter host cells, they 'hijack' plants and 'instruct' cells to produce more virus particles. As plant cells are converted from their normal function and processes (such as cell division or chlorophyll production), changes in plant growth and development may be observed.

Plant viruses do not move in and out of plant tissue as readily as fungal and bacterial pathogens. They require vectors (such as insects or humans) to carry them from one plant to another. After entry into plant cells, reproduction begins. Viruses spread throughout plant hosts, infecting all plant parts (systemic infection). Viruses are dependent upon live hosts for replication, thus disease progresses slowly. Virus-infected plants often survive for many years before they die, as rapid plant death would be detrimental to a pathogen that depends upon its host for replication.

Insect vectors are a common means for virus spread. Numerous insects are capable of acquiring virus particles while feeding on infected plants. These particles are transferred to new plants or plant parts during subsequent feedings. Some virus particles can enter an insect's gut where the virus may persist and replicate throughout the insect's life. Other types of particles are only carried for short periods inside probing insect mouthparts. Insects are capable of transmitting viruses multiple times throughout the season. Viruses can also spread

by infected seed or pollen, nematodes, humans, animals, or tools. A few viruses with very stable structures can remain viable in dormant plant tissue and on nonliving materials, which can serve as inoculum for future infections. Common symptoms caused by viruses include mottling, mosaic, leaf distortion, stunting, poor fruit set, and chlorosis.

Plant parasitic nematodes: Plant parasitic nematodes are microscopic roundworms that primarily infect roots, but a few occur in foliar portions of plants. While there are many species of nematodes, only a few are known to parasitize plants. All plant parasitic nematodes have needle-like mouthparts (stylets) that are used to pierce plant tissues and extract cell contents. Nematodes reproduce via eggs that result from either the mating of a male and a female or by the female alone.

Symptom development occurs as a result of extracted cellular contents or other plant damage. Nematodes may remain on the exterior of roots during feeding (stubby-root nematode) or penetrate plant tissues completely to feed while inside plants (dagger nematode). Nematodes may select a single feeding site or feed in multiple locations. Long distance spread is achieved via movement of infested soil, floodwater, or plant material; nematodes are only capable of moving very short distances on their own. Common symptoms caused by nematode feeding include chlorosis, root galls, damaged or stubby roots, stunting, dieback, and reduced yields. Some heavily infested herbaceous plants develop symptoms similar to nutrient deficiencies as a result of root loss.

Phytoplasmas: are extremely small bacteria-like plant pathogens. While they are similar to bacteria, phytoplasmas differ in their inability to survive without a host, their smaller size, and their lack of cell walls. Phytoplasmas rely on insect vectors, such as leafhoppers, for transmission into hosts. During feeding, leafhoppers acquire phytoplasmas from infected host phloem (nutrient-conducting vascular system) and introduce them into healthy tissue. Once in the phloem, phytoplasmas are capable of reproducing and spreading throughout plants, which results in systemic infections. Phytoplasmas persist within their insect vectors, making this the main method of spread for these pathogens. Common symptoms caused by phytoplasmas are virescence (development of chlorophyll in tissues where it is normally absent) and abnormal growth such as a 'witches broom' symptom. The most common disease caused by a phytoplasma is aster yellows.

Parasitic Seed plants: Parasitic seed plants share many common characteristics with true plants, such as their ability to reproduce and spread by seeds. Like other plants, seeds are

disseminated via wind, water, and animals. However, parasitic plants lack the ability to produce all of the nutrients or water they require, so they rely on host plants. In some cases, parasitic plants have developed modified structures, such as haustoria, that allow them to penetrate host plants and obtain nutrients and water. Common symptoms caused by parasitic plants are reduced vigor and dieback.

Some diseases caused by living (Biotic, Parasitic, or Infectious) agents:

Fungi; Bacteria and Phytoplasmas (formerly known as mycoplasmas or mycoplasma-like organisms); Viruses; Nematodes; Parasitic Higher (Flowering) Plants Several flower- and seed-producing plants live as parasites on other plants (host plants).

Some diseases caused by abiotic (Nonliving, Nonparasitic, or Noninfectious) agents

A variety of environmental and cultural factors can cause diseases in plants. Since these diseases occur in the absence of pathogens, they do not spread from a diseased plant to a healthy plant.

High or Low Temperatures; High or Low Soil Moisture; High or Low Light Intensity; Lack of Aeration or Low Oxygen Supply; Air Pollution; Nutrient Deficiencies; Mineral Toxicity; Unfavorable Soil pH; Pesticide Toxicity; Improper Cultural Practices.

Plant disease diagnostics: begins with the observation of both the symptomatic plant(s) and surrounding environment. Sometimes the cause of plant problems is evident: wildlife damage, insect feeding, or mechanical injury. However, a wide range of abiotic and biotic factors can cause disease or disease-like symptoms.

There are several steps to disease diagnostics, including evaluation of vital site information and examination of diseased tissue. Since the majority of plant pathogens cannot be seen without the aid of a microscope, it may not be possible to confirm disease agents by visual assessment alone. In order to diagnose plant problems, it may be necessary to submit samples of symptomatic plants to a university or commercial laboratory for further analysis.

Even though it may not always be possible to diagnose plant diseases by symptoms alone, the following strategies can be used to determine whether an infectious agent is the cause of disease symptoms. In addition, detailed site and plant information can lead to a more complete diagnosis.

Know the host plant(s): A single plant species can have numerous cultivars or varieties, with many different colours, shapes, patterns, textures, and sizes. Occasionally, diversity or variation in plant appearance can be mistaken for disease. The difference between normal growth and appearance and abnormal appearance can indicate whether a problem is present. Furthermore, disease symptoms may differ with plant species or cultivar. Some species and/or cultivars may be more susceptible or tolerant to disease than others. Host information can be critical for diagnosis.

Document plant part(s) affected: In some cases, symptoms develop on plant parts that are different from infected tissue. For example, dieback and wilting seem to indicate problems in the plant canopy. However, a root rot pathogen or bacterial colonization of vascular tissue may cause similar symptoms. It is important to examine all above and below ground portions of plants to determine potential infection site or symptom origin. Digging or cutting into plant tissue may sometimes be necessary to fully understand the extent of parts affected.

Check for symptoms and signs: The presence of symptoms often indicates some sort of plant problem. A wide range of symptoms can be expressed by diseased plants. This is further complicated when abiotic factors induce disease-like symptoms. For example, fungi and bacteria can cause leaf spots that are similar to those caused by herbicides and ozone. Plant disease diagnoses, therefore, cannot be based upon symptoms alone. Identification of

signs (mycelial growth, fruiting structures, and bacterial cells) with a hand lens or microscope is required to confirm diagnoses. Often, a trained diagnostician can assist with this task.

Examine the site: Examine the area around plants for additional clues as to the cause of plant problems. This is particularly important with abiotic problems. Information regarding soil make-up and disturbances, as well as soil compaction and drainage patterns, can assist in differentiating abiotic maladies from diseases. Additionally, examination of surrounding plants can provide vital information regarding disease spread. Patterns of injury or symptoms can give clues as to whether variety- or species-specific infections are possible, or whether a more expansive problem exists.

Ask questions: Ask questions and collect additional information that is critical in making diagnoses. More information can lead to a more accurate diagnoses. Consider these site specifics: Planting date and practices? Irrigation practices? Fertilization? Symptom development? Weather conditions? Site disturbances? Traffic or pedestrians. Pet and livestock habits? Herbicide applications (dates applied in relation to when symptoms were observed).

Diagnosis: After collection of site information and analysis of a symptomatic plant(s), it may be possible to diagnose some plant problems at this stage. However, if the cause cannot be determined, it may be necessary to submit symptomatic plant samples to a diagnostic laboratory.

Plant disease management: Disease management begins with accurate diagnoses. Diseases can only be managed once they are correctly identified. The best management practice is to avoid disease altogether. While disease prevention is not always possible, there are often recommended management practices that can be used to limit disease spread and subsequent infections.

Principles of plant disease management

There are five principles to disease management that focus on prevention and limit of disease spread. These techniques are most effective in combination.

Exclusion:

Definition: Prevent pathogen introductions to areas where they do not currently exist.

Common Practices: Quarantine, inspection, and certified disease-free plant material.

Avoidance:

Definition: Inhibit establishment of pathogens that exist in other areas.

Common Practices: Use certified disease free plants or seed, inspect plants before purchase or installation, reduce plant stresses, rotate crops, and avoid wounding.

Resistance:

Definition: Select plants with increased tolerance to pathogen(s).

Common Practices: Select seeds or plants with resistance to common pathogens. Consult a county Extension agent or reliable source for information on resistant cultivars.

Protection:

Definition: Implement steps to protect plants from infections.

Common Practices: Modify environment to prevent pathogen infections, remove alternate hosts, apply physical barriers, biological or fungicide treatments.

Eradication:

Definition: Limit pathogen spread once a plant is infected.

Common Practices: Remove infected plant portions, remove (rogue) entire herbaceous plants including roots, sanitation, fungicide applications to minimize inoculum (fungicides do not cure disease or eradicate pathogens).

Diagnosing Plant Disease Problems**A. Why Is Diagnosis Important?**

Whether in an effort to save existing plants or to prevent problems from recurring, it is important to know “What went wrong?” Diagnosis is the process of gathering information about a plant problem and determining the cause. Once the cause has been determined, it is then possible to recommend a solution or remedy. Diagnosing plant problems can involve considerable detective work. Sometimes there is insufficient information and other times, the primary cause of a problem is hidden by more obvious, but less-important, problems. Success in diagnosing plant problems depends on how much we know about the host plant, about the plant problems in general, and the quality of information obtained from the client.

B. Basic Steps in Reaching a Diagnosis

1. Identify the plant—The better your plant identification skills the faster you will be able to diagnose a problem. Most references on plant pests and diseases are organized by plant, so knowing the plant is the essential first step in using many reference books.
2. What is normal? Your familiarity with the normal appearance and cultural requirements of the plant will enable you to differentiate normal changes from symptoms of a problem.
3. What is the problem? To make a disease diagnosis, you need to know: the pattern of distribution of the diseased plants or plant parts, the plant species or cultivar involved, the site where the plant is growing (field, orchard, garden, greenhouse, inside the house, etc.), and previous crop history of the site. For example, uniform damage to many species in an area, to all plants on one side of the field or garden, or to all shoots on one side of the tree indicates that the cause may be an abiotic factor. Also, if the damage is well demarcated in a garden or in a plant, it may suggest that some abiotic factor is involved. On the other hand, if there is evidence of progressive spread of the disease from an initial focus to other plants of the same cultivar or species or to different parts of the plant, it may indicate that an infectious agent is involved.
4. Examine the plant and note symptoms and signs —For a presumptive diagnosis of diseases in plants, look for the symptoms and signs of the disease. The characteristic internal or external alterations of a plant in response to a disease-causing agent are called symptoms (leaf spot, necrosis, blight, canker, wilt, lesion, gall, witches' broom, rot, chlorosis, mosaic, etc.). Sometimes, the pathogen that causes the disease produces its own characteristic growth or structures on the diseased plant that are of diagnostic value. These are referred to as signs of the disease (mold, mildew, sclerotia, mushrooms, conks, etc.).
5. Tentative diagnosis—Based on your knowledge of the plant and information from reference books, formulate a tentative diagnosis. This will help you focus your examination of the plant and assist in collecting relevant information.
6. Double-check the diagnosis—Once you have arrived at a diagnosis, unless it is an obvious diagnosis, double-check it. Ask other master gardeners or extension educators for their opinions. Read through the reference books about your diagnosis to make certain everything matches. Additional laboratory work may be needed to confirm your diagnosis.

7. Types of plant disease diagnosis—Verbal descriptions by a telephone call or evaluation of a sample provide the most common diagnostic opportunities. However, a site visit provides more complete information. To make a telephone diagnosis, you must completely rely on information provided by the caller in order to make your diagnosis. There will be common, familiar problems, such as powdery mildew of apples, when a little information easily leads to a correct diagnosis. In other cases, it will be very difficult to make a diagnosis over the telephone and it may be necessary to evaluate a sample. Much of your diagnostic work as a master gardener will be done with plant samples. Usually, the sample will provide the clues necessary to solve the problem. But when the sample only confirms the identification of the plant, you must concentrate on acquiring information to reach a diagnosis. Your job will be to learn about the plant's cultural and environmental conditions, the care the plant has received, and whether the sample is representative of the problem affecting the plant. A site visit provides the greatest opportunity to gather information, but a successful plant diagnosis also depends on a combination of factors: your knowledge of the plant involved, your understanding of the plant's basic cultural requirements, and your recognition of the potential problems that might affect it. It also depends on your ability to gather information, both through observation of the plant and discussion with the client.



AGRICULTURAL PRACTICES AND ANIMAL HUSBANDRY

The branch of science which deals with methods of food production is known as agriculture. Besides studying the new methods of food production, in this branch of science we also study about how new and better varieties of crops can be grown, how animals and birds like cows, hens, etc. can be reared well and made to give more milk or better quality eggs? All these new methods which scientists develop come under agricultural practices. We need vegetables, fruits, cereals, pulses, etc. as food. For our clothes, we need the fibre of plants or animals. We get all these foods and fibres by farming or agriculture.

Vegetables and fruits are essential items of our diet and their growth and production are studied under a branch of agriculture called horticulture. Horticulture is derived from two latin words: hortus which means garden, and culture which means cultivation.

The branch of agriculture that deals with growing and production of vegetables, fruits, ornamental plants and management of orchards is called horticulture.

Horticulturists research to find new ways by which better varieties of fruits and vegetables can be grown in large quantities.

STEPS IN RAISING IMPROVED AGRICULTURAL PRODUCE

To increase our food production we can sow good quality seeds and improve the methods of sowing. We can make the soil more rich and even use better techniques for harvesting the crops. Some of the agricultural practices which scientists have developed and which farmers have started are explained below.

1. Preparation of soil:

This is an important practice which helps to enrich the soil and make it more fertile and aerated. It involves addition of manure followed by turning, loosening and levelling of the soil, using agricultural implements like spade, plough or mechanical farm implements.

2. Seed treatment:

Seeds can easily be attacked by micro-organisms. The crops that grow out of diseased seeds will also be unhealthy. So farmers treat these seeds by dipping them in certain chemicals. These chemicals do not allow the microorganisms to attack the seeds and damage them. Such chemicals are called Fungicides. Once the seeds are treated, they can be sown.

3. Preparing the seed bed and care of the seedlings:

In certain crop plants like paddy and some of the vegetables, seeds are not sown directly in the main field. First these seeds are sown in a nursery bed. Once they grow to a certain age they are transferred and planted in the main field. These small plants are called seedlings.

When the farmers prepare a nursery bed they take care of the following:

- The soil of the bed should be soft and loose so that the tender roots of the seedlings can grow well. This can be achieved by digging or ploughing the field well.
- The seed bed or where the seedlings are planted should be even so that when we water the plants, the water distributes itself uniformly all over the field.
- All weeds or unwanted plants in the field must be removed. Do you know why? It is because these weeds also take water and nutrients from the soil and as a result the desired plants cannot get enough of the nutrients. The seedlings

also need to be protected from diseases and pests. This is done by spraying chemicals on the seedlings.

4. Transplanting:

The process of removing the seedlings from the nursery bed and planting them in the main field is called transplanting. When we transplant, we must select those seedlings which have 4 to 5 healthy leaves. These are sowed at proper distance from each other. The main field must be ploughed and manured before transplanting. Generally rice and vegetables like tomato are sown by transplanting. Transplanting of seedlings is a very important practice. This enables us to select good and healthy seedlings and get a better crop. Besides, when we transplant seedlings, their roots are able to go deep into the soil and get more nutrients. When seedlings get good food, they grow into healthy plants and give a better yield.

5. Adding fertilizers:

Crops need nutrients like phosphorus, calcium, nitrogen etc. for their growth and pick up these nutrients from the soil. It is very important to add fertilizers to the soil. They provide nutrients to the soil and help to obtain a better crop yield. Depending on the type of soil and the crop to be grown, we use different fertilizers. The way we use a fertilizer also depends upon what type of fertilizer is being added to the soil. A fertilizer which contains nitrogen (nitrogenous fertilizer) is generally given in two or three doses. Other fertilizers are phosphatic and complex fertilizers. Some fertilisers are added to the soil before transplanting.

While fertilizers are manufactured from chemicals in factories, manure is made from organic substances and contains nutrients in small quantities. Some of the commonly used manure are: Farmyard manure, as the name suggests is a mixture of decomposed cattle dung (excreta) and urine, left over fodder (cattle

feed) and litter (bedding provided to cattle in the farm). Compost is manure made from vegetable and animal refuse collected from domestic waste, straw, weeds etc., dumped in a deep pit to decompose.

6. Use of plant growth regulators:

Plant growth regulators are certain chemicals which regulate the growth of plants. All plants have growth regulators which determine how tall the plant would be, how big its fruit will be, etc. We can now add some plant growth regulators to get a better yield of crops.

7. Irrigation:

Irrigation is necessary for proper development of plants. Roots fail to develop and penetrate in the dry soil. The crop is irrigated according to its requirement and soil characteristics. Irrigation is essential during the seedling, flowering and grain filling stages of the crop. Rice crop needs standing water.

8. Harvesting:

Harvesting machines have now replaced the back breaking job of hand harvesting with the sickle and scythe. Harvesting machines cut or dig out the plant or its parts as required. The machines gather the plant parts, separate desired parts and eliminate parts not needed. Certain harvesting machines may even load the crop for transport. However, the above mentioned functions of harvesting machines depend on kind of crop, plant parts to be harvested, crop use, stage of maturity, etc.

SOME OTHER DIFFERENT AGRICULTURAL PRACTICES

1- Rotation of crops:

The process of growing a different crop preferably a leguminous crop in between raising of two similar crops is called rotation of crops. Crop rotation has a lot of benefits, (i) the land gets utilized, (ii) the pulse crop uses up different nutrients from the soil but it fixes the nitrogen from the air and makes the soil richer in nitrogen and so more fertile. This way the next cereal crop gives a better yield. If we do not practice crop rotation by growing different crops on a piece of land, but continue to grow the same crop, year after year, they will keep on using the same nutrients from the soil till all the nutrients in the soil get used up. The newly grown plants get poor nourishment from the soil and grow up to be weak and of bad quality. When plants are weak the insects can easily attack them and destroy them. Thus, crop rotation restores the fertility of the soil, it gives better yield, prevents crop from diseases and pests and reduces the dependence on chemical fertilizers.

2- Multiple cropping:

Growing two to four crops one after the other in a year in the same field is called multiple cropping. Multiple cropping is possible, when we make use of crop varieties that grow for a short period of time. However, to get best results there must be a properly managed field. In fact, multiple cropping is the best solution for a country with food problem because same piece of land is used to grow different kinds of crops.

3- Improving the variety of seeds:

You must have often heard or read advertisements which encourage farmers to buy new and better varieties of seeds. Some of these new varieties are resistant

to diseases and give a very good crop. Some of the improved high-yielding crop varieties which our scientists have developed (Rice, Wheat, Maize).

Do you know what name is given to scientists who develop such new varieties of seeds? They are called plant breeders. Plant breeders have not only raised better quality seeds but also better quality fruits.

PROTECTION OF CROPS IN THE FIELD

As crops grow in the field, they have to be protected such that they produce a healthy yield. The weeds growing along with crops have to be removed and growing crops have to be saved from the attack of pests especially insects pests.

1- Weed control:

The undesirable plants that compete with the main crop for sunshine, water and space in the field are called weeds. Weeds must be removed as they use up the nutrients in the soil and thereby make them unavailable for the crop itself.

Before sowing or transplanting seedlings, weeds are removed by hand or with the help of a plough or harrow. If some of these weeds start growing again during the crop growth they must be removed. They can be removed by hand or by spraying weed killing chemicals called weedicides.

2- Control of plant diseases and pests:

A lot of plants die due to some diseases and pests which attack and damage them. Generally plant diseases are caused by fungi, bacteria, and viruses. These diseases are transmitted either through the seed itself (seed-borne) or by air (airborne) or soil (soil-borne). Insects are generally pests which eat and destroy crops. To control plant diseases and pests we can spray fungicides and pesticides on the crops or on the soil. Apart from chemical methods, biological

control methods are also used. For example, some insect pests are controlled by introducing their predator insects.

ANIMAL HUSBANDRY

The branch of science, which deals with the study of various breeds of domesticated animals and their management for obtaining better products and services from them is termed animal husbandry (the term husbandry comes from 'husband' which means one who takes care).

- Milk giving animals: Cows, buffaloes and goats who give us milk.
- Meat and egg giving animals: cattle, goat, sheep, fowls and ducks which are the main source of meat. From hens and ducks we get eggs.
- Working animals: Bullocks, buffaloes, camels and horses are draught animals used for doing work in the field and for transportation of goods and human beings. Mules are also used especially by the army to take things from one place to another in the hilly areas. We also get horns, feathers and leather from some of these animals which can be used for making various things. Their urine and droppings help to make the soil fertile by acting as manure.

a- Need for animal husbandry:

We have a large number of animals in the country. Yet we do not get as much food from these animals as we possibly can and need for our large population. Besides the food, which we get from animals, we need them to do a lot of our work. For example, in India, If we take the ratio of working cattle to the area of land, which is being used for cultivation we find that only two individuals of cattle are available to plough 3.8 hectares of land.

You all know that cattle wastes like urine and faeces are natural manure which enrich our soil. Animal husbandry is a very important field which helps us to improve our livestock and other useful animals and make the maximum use of them.

b- Management of livestock:

When we study about improving our livestock we learn how they must be sheltered, fed, and mated, what kind of drinking water should be given to them and how the sick and diseased animals ought to be treated? This way we learn to manage our livestock for better production and utilization.

1. Feeding of animals:

All animals must be fed properly. The food should contain the requisite nutrients i.e. carbohydrates, proteins, fats, minerals, vitamins and water. The food which is given to cattle can be divided into two categories:

- Concentrates like cotton seeds, oilcakes, cereal grains, bran etc. They are very rich in most of the nutrients.
- Roughage includes fibrous and rough food like straw and stems of cereal crops. Generally roughage has a low nutrient content. Cow eats green fodder and dry grass, which is mixed with a sufficient amount of grain. Goat and sheep eat grass, herbs and waste products from the farms. Poultry birds are given a mixed feed consisting of cereals, bone meal, minerals and vitamins.

2. Housing of animals:

We must protect our animals from too much heat, rain and cold. We must, therefore, be careful where we house them. Their houses should have proper sanitation and ventilation. Too many animals should never be kept in a small space. Different animals require different types of houses. Hens and fowls are

kept in cages while sheep and goats stay in open yard, which is partially covered with roof made of straw. This open yard should have a hedge of iron wires all around to prevent the animals from running away.

3. Water and its supply:

To keep these animals healthy they should be given clean water to drink and in sufficient quantities. For example, on an average a cow consumes about 27-36L of water, camel 8-90L and poultry birds require about 240mL of water. Besides this we must also bathe the cattle with clean water.

4. Some common diseases of animals and vaccination:

Sometimes domestic animals may be afflicted by diseases. Some of the common diseases of animals are listed below.

Viral diseases: Pox in cattle, goats, sheep, and fowls; dermatitis in goats and sheep; foot and mouth disease in cattle.

Bacterial diseases: Tuberculosis in cattle and poultry birds; cholera in fowls; diphtheria in calf; diarrhea in chicks; foot rot in sheep.

Most of these diseases can be prevented by proper sanitation, a controlled diet, proper housing and also by vaccinating the animals against these diseases at the proper time and age.

5. Animal breeding:

Breeding means to reproduce. In case of animals, breeding is done to obtain animals with desired characters. The two individuals of desirable characters can be selected as parents. These are then crossed to obtain new breeds of animals, e.g. by cross breeding a cow of low milk yielding breed, we can get breeds of cow which produces more milk.

Artificial insemination is an important and effective method of breeding. The process involves injecting the semen obtained from desired bull belonging to high milk yielding breed into the reproductive tract of female during heat period. It generally gives important breeds, and is widely used to improve the qualities of cow, buffaloes, poultry, horse and goat etc.

Over the last two decades, improved practices of raising animals have resulted in the development of new breeds of dairy animals, poultry. This has substantially increased our milk, egg and meat production.



Hen Basics

Facts:

- Chickens are ground dwelling birds, but can fly for short distances. They are very social creatures that depend on one another for companionship and security. Chickens can live 14 years or more.
- Layers need approximately 15 hours of light a day in order to keep laying. You may wish to provide an artificial light source during the darker winter months or you may wish to give your hen the natural break during the winter.
- Egg laying for a hen generally starts at 6 months old. Domesticated hens have been bred to lay one egg a day, but by 18 months of age, egg laying generally diminishes and many adult or senior hens stop laying altogether. Purebred chickens or heritage breeds tend to lay eggs longer.
- The majority of a hen's waking hours are spent active, grazing, foraging for food (plants, bugs and occasionally small rodents), dust bathing, preening, playing and napping.
- Chickens are sociable creatures.

Needs & Behaviour

- ✓ Hens need living space that is dry, draft free and provides safety from both predators and pests.
- ✓ Chickens are social animals. Generally, 3-5 compatible chickens can be well maintained in a typical city environment. Individual bird's age and temperament can affect compatibility. Overcrowding, boredom and barren pens are the most common cause of behavior problems.
- ✓ Taking a dust bath is the closest thing to heaven for a chicken. They bathe in the sun and in loose dry soil depressions in the dirt which cleans their feathers and helps rid them of parasites. Birds will usually dig their own hole for dust baths. If they are unable to dig their own dirt bath area you will need to provide one. Keep the soil in the dust bath loose and add play sand if it is a heavy clay soil. Adding a little poultry dust, diatomaceous earth or wood ash increases the effectiveness of parasite control. Large heavy rubber feed buckets filled with play sand are a welcome addition to the indoor coop in the winter.

Costs

- Besides construction of a chicken coop and pen, and purchase of the hens start up costs also include food storage, security locks, lighting, and feed & water dishes.
- Ongoing costs include building maintenance & cleaning, bedding, food, nutritional supplements, parasite control, grooming, vet care, and care when you are away over a longer period of time.

The following information was compiled from a list of sources provided at the end of this document. This information should be used as a starting point for understanding the best management practices for the keeping of chickens in an urban area.

Humane treatment

- ❖ Never handle a chicken by wings, feet or legs. Herd birds to a corner using slow deliberate movement. Fast=predator, slow=less threat. Place hands over top part of wings (shoulders) and hold securely but do not squeeze too hard. Pick up and hold under arm to keep wings in place. Support feet with other hand if bird will tolerate. To restrain for transport or examination, drape a towel over shoulders cape style and wrap around body.
- ❖ Are they breathing heavily? Check that they have enough shade to find a spot where they can cool off.
- ❖ Are they losing feathers? The proverbial “pecking order” may be in effect. If any of the chickens are bleeding, consider separating it, as other chickens will continue to peck at the wound. Loss of feathers could mean that a hen is moulting. Moulting is the natural shedding and renewal of feathers and occurs about once a year. It is a difficult time for birds, since it involves hormonal fluctuations and increased energy requirements. Moulting normally occurs at the end of an intensive laying period and can take about seven weeks for the new feathers to complete the growth cycle.

Feed

- Feed supply stores are a good source for pelleted poultry feed (for large breeds) or crumble (for bantam breeds). You can get regular or organic, just make sure it is the larger pellets for adults or a starter mix for chicks.
- Always make sure the food and water are fresh, cool, and clean.
- Put the feed in a covered feeder and replace it regularly, as it does go stale. Never let your chickens eat damp feed! Chickens also love table scraps.

- Food receptacles should be made of non-corrosive material that is easily cleaned. This minimizes spillage, prevents contamination with droppings and keeps food the dry. The containers should be large enough for all the birds to comfortably eat at once or numerous enough to prevent competition or intimidation.
- They are effective weed eaters and insect control. Fresh and cracked corn is a favourite as well as tomatoes, apples, anything baked, and whole grains.
- Steer clear of giving your chickens onions and garlic, as it can flavour the eggs.
- Chickens are inquisitive and voracious eaters of vegetation and many ornamental garden plants can be toxic to them. Learn which of your plants might be harmful and be sure your hens cannot reach them through the fencing.
- Clean up uneaten food and any kitchen scrapes that you may give them soon after they feed in order to avoid attracting nuisance animals.
- Also provide crushed oyster shells as a source of calcium.

Water

- Water receptacles should be made of non---corrosive material that can be cleaned and disinfected with a solution of chlorine bleach, prevent contamination with droppings and is spill and leak proof.
- The containers should be large enough for all the birds to comfortably drink at once and hold enough water for all birds for an entire day.
- Food should be slightly positioned higher than the water or far enough away to prevent contamination with food.
- In the cold weather you will need to ensure that their drinking water does not freeze.

Health Basics

- ✓ Prevent contact with wild birds and other animals.
- ✓ Clean the coop regularly.
- ✓ Keep the coop dry and ventilated.
- ✓ Keep new and returning birds separate from the rest of the flock for a period (approx.10 days).
- ✓ Be on the look out for signs of disease. Some possible symptoms to look for indicating injury/illness/disease include: Coughing, wheezing, labored breathing, warts/scabs, swollen joints, loss of feathers, reduced egg production, thin egg shells, fever, enlarged abdomen.

Introducing new hens:

When bringing in new birds a 2 week quarantine in a separate area is recommended to watch for signs of illness and parasites. Avoid noisy, high traffic areas and allow the bird to acclimate before introducing to other birds, animals and family.

Chicken coop:

- The key is to make your coop a safe, dry and draft free place for the chickens to lay their eggs, roost, eat, drink and sleep.
- The coop needs to be a closed structure which provides shelter from cold, heat, wind, rain, snow, and predators.
- Coop and pen should be located in an area that provides shade, direct sunlight, good drainage and protection from prevailing winds and will not present a problem to neighbours.

Ventilation and Light:

- ❖ To stay healthy, your chickens need plenty of ventilation and light.
- ❖ Chickens have a thick coat of feathers to keep them warm, but they are vulnerable to poor air quality and pathogens in the litter.
- ❖ Chickens may be unwilling to eat in the dark which means they can starve even if there is plenty of food available.
- ❖ Doors, windows and vents near the ceiling supply oxygen, remove heat, harmful gasses and dust particles, and dilute disease causing airborne organisms. However, if they are huddling together in a particular spot it could be the sign of a draft at their level that will need to be blocked.
- ❖ Design and construct your chicken coop with good ventilation through the roof eaves and windows that open making sure that openings are pest and predator proof (cover with at least ½ inch metal screen). Windows or skylights will provide the light they need.
- ❖ To keep your chicken coop cool on hot summer days locate your coop in a shaded area.

Doors

- Doors for chickens should be just large enough for the largest bird and can be positioned anywhere from ground level to about 2 feet high with a stable ramp with cleats.
- Doors must be able to be secured against predators at night.

- Do not forget to provide easy human access so you can collect eggs and clean the entire coop.

Bedding

- Regardless of the floor type, bedding material strewn on the surface is needed to absorb feces and spilled water to keep the chicken's feet dry.
- Material for the floor should be clean, non-toxic, biodegradable, and easily replaceable.
- Play sand, leaves, straw or hay, shredded paper, or wood shavings work best and can be composted.
- It is important to rake out the bedding and replace it frequently to reduce odor and fly activity.
- Make sure that the bedding material is never left long enough to become moldy.

Floor

- ✓ The floor should not collect and hold moisture. It should be easy to clean, be able to retain heat in cold weather and keep out rodents and predators.
- ✓ A wood floor is adequate provided it is at least a foot off the ground, insulated and sealed properly, but wood is difficult to sanitize because it is porous.
- ✓ A concrete floor discourages rodents and predators and is easy to sanitize.

Roost

- Chickens need somewhere for the chickens to perch at night. Determining the right chicken coop roost height is important in keeping your chickens comfortable and healthy.
- Chickens do not usually sleep on floors. Their nature makes them look for an elevated place to sleep to protect themselves from predators.
- The best material to use for perches or roost bars is wood. Lumber or branches can be used. They should be strong enough and mounted securely enough to hold all birds. The surface should be rough for good grip with no splinters or sharp edges.
- For medium sized birds, 2" or 4" flat or 1 1/2" diameter is best for foot comfort.
- The longer the roost the better to prevent competition.
- They should be set 18" from wall, 2-4 feet off the floor. If mounted higher, rung steps are needed spaced 8-12 inches apart.

- You may wish to provide removable litter boxes or planks directly below the roost for easy cleaning.

Roof

- ❖ The coop roof should be made of a material that will not collect and hold heat.
- ❖ The roof surface should at least be covered with an insulating tar paper to protect from heavy rains.
- ❖ The roof should be slightly inclined, to allow water to run off and if it overhangs it will protect from downpours.
- ❖ A few small openings along the eaves allow moisture to escape and provide fresh air.

Nest box

- Provide one nest box for every 2-3 hens.
- The boxes should be filled with 2-4 inches of straw, wood shavings, litter or grass.
- Chickens prefer wooden nest boxes with covered opening for privacy.
- Place the boxes on or as low to the floor as possible. If set higher they require a perch in front of the opening.

Cleaning

- A good hosing off of the coop once a year with a spray of a vinegar or environmentally friendly cleaning solution is required.
- Once every week or two (depending on how many chickens you have in how little space), clean out the coop, wipe down the perches, and scrub down their feeders.
- If the chickens are laying, thoroughly clean their nest boxes, especially if an egg cracked at one point.
- Keeping their environment clean will also keep your neighbors from complaining.
- Chicken manure does have an odor of ammonia so it does need to be absorbed by bedding. Cleaning out the bedding will keep the odor down bedding can be put in a compost.

Outdoor Run

- ✓ The fenced area surrounding the coop that provides secure access to exercise, sunlight, earth and vegetation and is freely available to the birds when they are unsupervised during the day.

- ✓ Give your chickens room to roam, give them a good spot to get out of the weather and up off the ground.
- ✓ It needs to be constructed to prevent the bird's escape and prevent entry by intruders/predators. Predators of chickens in an urban area include: dogs, cats, and raccoons. It's best to build the strongest deterrents possible.
- ✓ Don't use chicken wire, as it is too flimsy and is easily torn into.
- ✓ Chain link panels or welded or woven fencing on a sturdy frame reinforced at the bottom is better.
- ✓ Protect against burrowing vermin such as rats, mice or raccoons by burying about 6 inches of the fencing wire below ground level and curling it outwards. Then when pests try to dig under the wire to get in your coop, they run into the wire instead.
- ✓ The outdoor pen must also be enclosed to keep hens in and predators out. Covering the pen with a roof such as corrugated fiberglass can provide shade and rain shelter.
- ✓ Regular inspection is key to security.
- ✓ Providing a wind and rain/snow break will give the birds a protected area to be outdoors even in winter.
- ✓ If the coop is elevated 2 feet so the chickens can use the space underneath, the coop and pen should not occupy the exact same footprint.
- ✓ Do not forget to provide adequate opening and space for a person to be able to clean the entire outdoor pen area and to be able to catch hens if needed.

The preservation of food

Why does food go bad?

Food contains the proteins, fats, carbohydrates and vitamins which are vital to life. It should be fresh when we eat it. If it is bad, it can make us ill. There are two main agents which turn food bad: fungi such as yeast and various moulds, and bacteria. These are micro-organisms which cannot make their own food and which live and grow on our food. Moulds, for example, are often seen on old bread. Yeast can spoil fresh food but it also has some very useful properties. For hundreds of years it has been used by man in the making of bread and wine. It acts as a catalyst in the process of fermentation.

In order to grow and multiply, all these micro-organisms need food, water, warmth and, in some cases, air. The methods used to preserve our food are intended to make conditions dry and very cold, unsuitable for the growth and multiplication of micro-organisms.

The great distances which often separate the producer of food from the consumer in the 20th century make effective food preservation vital. But in most preservation processes, many important vitamins and proteins are wholly or partially destroyed. One of the tasks of food technologists today is to find ways of preserving without losing these vital substances.

How is food dried?

In hot countries, food is dried simply by the heat of the sun. The moisture level in most fruits can be reduced to between 5% and 15%, which is low enough to inhibit the growth of micro-organisms. Some other foods are subjected to a process known as dehydration. In this process, a current of hot, dry air is passed over the food to absorb as much moisture as possible. Tea and coffee are often dried in this way.

What happens to foods when they are canned and bottled?

High temperatures kill micro-organisms in food and most micro-organisms need air. That is why food is vacuum sealed in cans and bottles and then heated up to a temperature of 100°C (acidic foods) or 120°C (non-acidic foods) for about 10 minutes. The food will then be kept for long time provided that the can or bottle remains airtight.

How else can food be preserved?

There are several other ways of preserving food. One of them, freezing, will be dealt with later. Two very old methods, salting and smoking, are still used today.

A concentration of 5% or more of salt in food inhibits the growth of most micro-organisms. Smoking causes partial dehydration. Certain acids and chemicals are useful preservers as they stop the action of the enzymes produced by micro-organisms. Vinegar, for instance, is used to preserve onions and other vegetables. One of the newest methods is radiation. It is especially effective because it kills not only micro-organisms, but also their spores, thus stopping their reproduction.

Refrigeration and refrigerators

Why is quick-frozen food almost as good as fresh food?

The micro-organisms which spoil food cannot grow or multiply at sub-zero temperatures. Most of our food consists of animals or plants cells containing a high proportion of water. If the food is frozen gradually, the water expands and forms large crystals of ice. These crystals burst the cell membranes and destroy the structure of the food. When the ice melts, the water runs out and most of the essential vitamins and proteins are lost. If the food is frozen quickly, smaller ice crystals are formed and the cell membranes are not broken. The greater the water content of food, the more difficult it is to freeze successfully.

How is food frozen?

There are three ways of freezing food quickly. It can be done by placing the food on metal plates which have been cooled below -30°C . It can be frozen by placing it in an insulated container and blasting it at high speed with air cooled below -30°C . Or it can be cooled in a salted liquid such as brine at a temperature below -30°C and -40°C . It must then be stored at a temperature below freezing point until it is needed. This is why insulated containers must always be used for the transport of frozen food.

How long can frozen food be kept?

Most packets of frozen food carry information about maximum safe storage periods. Most refrigerators and freezers have stars on them to show the minimum temperatures they can reach. Food should never be re-frozen after it has been thawed out as it can never be quick-frozen in a home refrigerator.

The table below gives the safe storage periods for various food products.

Food	Period in months	
	at - 18° C (0° F)	at - 12° C (0° F)
Fruit juices, beef	12-15	6-8
Fruit, vegetables	10-12	3-6
Lamb, poultry, eggs	8-10	3-4
Butter, cheese	6-8	2-3
Beef-liver, cooked food	2-4	1-2

How does a refrigerator work?

Many modern homes have refrigerators and freezers. They work on a principle of heat absorption. Certain gases, such as ammonia, condense and re-evaporate quickly when the pressure on them varies. These gases can be used as cooling agents in refrigerators. They are contained in a partial vacuum inside the evaporator. Compression causes the cooling agent to evaporate, to boil and to absorb the heat of the food in the refrigerator. The air and the metal walls of the evaporator act as conductors of heat in the course of the absorption. The cycle of the evaporation and the condensation continues as long as the refrigerator is switched on. The temperature is controlled automatically by a thermostat switch.

Animals in danger

1. Introduction

Fossils tell us that animals, such as fishes, reptiles and large mammals, lived on earth many millions of years before man. Later, early man's life was always threatened by the huge animals which roamed the world. Today, the situation is reversed. Many wild animals are threatened by man. In the press, attention is most often concentrated on the problems of the whale, but many other animals are in danger, too. Here is some information about three of them.

2. The American bison

In the year 1700, about 60 million bison wandered freely in North America. Their range was very wide and they were still multiplying despite being hunted by Indians. Today, the bison population of North America is around 10,000, a mere fraction of the former total. How has this terrible decimation come about? The white man and his rifle must take the blame. During the building of the Union Pacific Railway, the bison was hunted more for pleasure than for its meat. By the 1890s, only a few hundred bison were left. The recent increase in numbers has been made possible only by strict government control over hunting. Now the bison is threatened more by infectious disease than by hunters. Its breeding areas are all protected.

3. The tiger

The tiger has suffered badly from its reputation as the enemy of man. It has been hunted so extensively that there are now probably fewer than 1,000 in the wild. Members of the tiger population have decreased in proportion to the expansion of human population in areas where it used to roam freely. The

human population of Asia has grown so dense that the future of the tiger looks black. Soon the only tigers may be in zoos.

4. The komodo dragon

The Komodo dragon is the world's largest lizard. It was not discovered until last century and was named only in 1912. It was formerly found on three Indonesian islands: Komodo, Rintja and Flores. By the 1930s, in spite of government protection, the population of this great hunting lizard had been reduced to a few hundred. It was, however, still found on the three main islands of its range and there were still individuals of over 4 metres in length. By 1963, after an interval of only fifty years since its discovery, almost the entire population was based on Komodo. It is now unusual to see one as long as 3 metres. The dragon's normal diet consists mainly of deer and wild pigs, animals which the islanders also hunt. This, of course, means that the dragon often gets too little to eat. It will certainly die out soon unless its hunting and breeding grounds are protected more effectively.