

MARVELS OF INSECTS

The Oil Palm Pollinating Weevil

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The Oil Palm or "Katupol" in Sinhala, botanically known as *Elaeis guineensis*, is an oil-producing plant found in a wild, semi-wild and cultivated state in the equatorial tropics. Large areas in Africa, South East Asia and South America have been planted with oil palm. The principal oil extracted from the fruit of oil palm is commonly called palm oil and has a major share of the edible oil market in the world.

Compared to other oil producing plants such as coconut, ground nut and soya bean, oil palm is the most efficient oil producing plant since its production from a unit area of land is the highest. The average oil production in oil palm is 1570 kg per hectare, as against 1015 kg in coconut and 140 kg in soya beans.

The oil palm is a large perennial palm somewhat similar to the coconut palm but with a thicker crown of leaves. The Sinhala name Katupol aptly describes the rather vicious thorns, more like small spikes, found in the fronds. The male and female flowers are produced separately, and the fruits, which are deep red to purple in colour when ripe are slightly smaller than an arecanut. The fruit produces two kinds of oil. The outer fibrous portion of the fruit yields palm oil, which is the major commercial product. The small kernel in the hard-shelled nut yields palm kernel oil, similar in composition to coconut oil.

Oil palm appears to be indigenous to West and Central Africa. Large extents have been planted in West Africa, South America, Malaysia and Indonesia producing the major portion of palm oil found in the world market. Oil palm had been introduced to Sri Lanka in the late 50's, and a plantation of over 1000 hectares has been established at Nakiyadeniya in the Galle district.

This plantation, currently under the management of the Sri Lanka State Plantations Corporation has its own

factory to process the fruits and produce palm oil which is used locally for a variety of purposes. The area under oil palm is on the increase, with new areas otherwise unsuitable for traditional plantation crops being converted to oil palm. There are now several smaller satellite plantations around Nakiyadeniya.

As stated earlier, the male and female flowers are produced separately in the same plant, but at different times. In order to produce fruits, it has therefore been necessary to artificially pollinate the female flowers. Firstly, ripe male flowers are cut and pollen in the form of a fine powder is collected. Pollen is then manually dusted on to the female flowers to pollinate them. A large work force had been used for these tedious processes of pollen collection and dusting them on to female flowers. To dust the female flowers, it is necessary to climb the trees and get inside the crown full of thorny fronds, which is rather hazardous, for the thorns can indeed be very painful. A work force of over 100 had been employed daily for this uninteresting, tedious and hazardous job.

Quite recently, an insect scientifically known as *Elaeidobius kamerunicus* was discovered in the Cameroons actively pollinating the female flowers of oil palm. This insect is a weevil, similar in size and appearance to the common flour weevil, 'gulla', with a snout which is a characteristic feature of weevils.

These weevils feed on pollen grains, found abundantly in male flowers. When the weevils visit male flowers for feeding, the pollen grains also get lodged in various body parts of the weevil, particularly on the legs and the snout.

A large number of tightly-packed female flowers, each about the size of a small arecanut, is found in a bunch, which weighs about 7-10 kg. Sometime afterwards, the female flowers become receptive for pollination, and

discharge a characteristically scented nectar. The nectar is identifiable by humans if one smells a female flower when it is receptive, but the weevils can identify it at quite a considerable distance. The weevils, which have been feeding on pollen grains, then visit receptive female flowers attracted by the scented nectar, and during their feeding and explorations, deposit pollen grains on female flowers thereby pollinating them.

A few insects were imported to Sri Lanka and were kept in quarantine in the laboratories of the Coconut Research Institute. During the period of quarantine, a large number of tests were done to ensure that there were no 'unwelcome' insects and to ascertain that they only fed on pollen of oil palm. This test was particularly important as there should be no damage to other economically important plants. Of course, tests in other countries have shown clearly that this weevil is very specific to oil palm and that it does not damage other plants.

After a series of rigorous tests, the weevil was found to be 'pure' (fed only on oil palm pollen). Then began the multiplication of the weevil in the laboratory, which was a fairly straight forward task. The female weevil, after feeding on pollen, lays eggs inside male flowers. The eggs hatch out and tiny grubs come out. These grubs feed on male flowers and develop into adult weevils, after passing through several stages of the life cycle. It takes about 10 days from egg laying to the emergence of an adult weevil.

A few hundred laboratory-bred adult weevils were released at the Nakiyadeniya State Plantation on oil palms with freshly-opened male flowers. As soon as the weevils were released, they were seen flying excitedly near the cluster of male flowers and alighting on them. They were then seen slowly walking over the male flowers, and after sometime, were white in appearance for they were completely covered with pollen. The cluster of male flowers (also called inflorescence) which were tagged at the time weevils were released was examined 10 days afterwards.

We were jubilant to find the grubs in the now withered male flowers indicating that the weevils had laid eggs, which was evidence of their acceptance of the local conditions and establishment. At that time, the weevils were seen hovering around receptive flowers and feeding on the sweet nectar. To our joy, it was obvious that the weevil was going to do what it was supposed to do.

The progress of the establishment of the weevil and its spread in the vast plantation were monitored by frequent visits to the plantation and examination of inflorescences. Within a matter of two months, the weevil had spread to a distance of 1.5 km (about 1 mile) from the point of original release and after about four months of the release of the first consignment of weevils, the entire plantation was 'infested' with it.

When it was evident that the weevil was established in the entire plantation, manual pollination was stopped.

The fruits of this operation are now visible. The weevil is a vastly superior pollinator than the human being. It not only pollinates the superficial female flowers in the bunch but also goes into the crevices in the bunch thereby pollinating those female flowers which are lodged deep in the bunch that are inaccessible to human beings during artificial pollination.

Termination of manual pollination has saved several million rupees annually. The work force that has been employed in manual pollination can now be redeployed for more productive and less hazardous work. Not only that, since the weevil can crawl freely, they pollinate those flowers that are deep inside the bunch resulting in an increased number of fruits thereby increasing the palm oil production significantly.

The marvels of insects are yet again demonstrated. The weevil now does a job earlier done by human beings, much more efficiently and at no cost to us, not only at Nakiyadeniya but also at other satellite oil palm plantations bringing in more income to the state.

VIRAL PATHOGENS OF PLANTS

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Virology, the science of viruses was born in 1892 with the scientific study of tobacco mosaic virus (TMV), the 'Grand Daddy' of all viruses. However existence of viruses can be traced back for thousands of years; small pox virus has been described by Chinese in the tenth century before Christ. The earliest record of a plant virus disease dates back to about the middle of the 16th century when a reference was made to 'colour breaking' in tulip flowers.

1. Viruses: Living or Non-Living

The first solid awareness of the true nature of viruses came with the isolation of tobacco mosaic virus (TMV). Viruses can be precipitated out of suspensions by substances like ammonium sulphate which are protein precipitants. Many viruses can be crystallised eg. TMV form needle-like crystals, each crystal containing millions of TMV particles. The crystallised viruses will retain their ability to infect. Proteins of viruses can be separated from the nucleic acids by gentle separation methods such as using detergents or acetic acid. The separated nucleic acid retains the ability to infect. The separated nucleic acid and protein can be re-assembled or re-constituted into whole virus particles, which are infective. Crystallisation is a property the viruses share with molecules. Reconstitution of viruses too is a physical and a crystallisation-like phenomenon. In these respects viruses do seem and behave like macromolecules. Crystallisation, separation and reconstitution of viruses while retaining infectivity posed many questions concerning nature of life.

Minimal structural features of a cell include a cell membrane, cytoplasm, nuclear region or nucleus and an energy generation system. Viruses do not have the minimum requirements of a cell and are therefore acellular. Viruses do not have the ability to acquire and utilise nutrients, to grow, or to excrete waste. They do not metabolise, nor do they respond to stimuli. Viruses

therefore do not possess the attributes of living organism.

However, when a virus is introduced into a susceptible living cell the virus takes over the normal machinery of the cell and direct the cell to synthesise virus particles at the expense of the host's cell material. On infecting a cell, one single virus particle can produce 100,000 new virus particles. Viruses can mutate. On these counts viruses are like biological organisms.

Are these agents living organisms or non-living molecules? The capacity for reproduction and mutation is a property possessed only by living organisms. Yet they are like perfect inert matter in other aspects. Not only do they possess a dual nature but they also express one aspect of their nature in one situation and the other aspect in another situation. On their own outside a living cell, viruses are simple giant dead molecules. But inside a living cell they are obligate intracellular parasites with a great capacity to reproduce. Viruses therefore lie in no man's land between the chemist's molecule and biologist's organism. We cannot but wonder the aptness of a remark made over 2000 years ago by Aristotle "Nature makes so gradual a transition from the inanimate to the animate kingdom that the boundary lines which separate them are indistinct and doubtful".

2. Morphology and Structure

Viruses are too small to be seen with optical microscopes, and hence are referred to as ultra-microscopic. They are studied with the aid of electron microscopes. The size of viruses range from 400 x 250 nm to 17 nm. The largest viruses are the pox viruses which attacks the higher animals including man and measure 400 x 250 nm. Probably one of the smallest viruses is that which attacks the tobacco plant causing tobacco necrosis disease and it measures 17 nm in diameter.

There is a great variation in the shapes of viruses. They can be rod-like, near-spherical to octahedral, filamentous, bacilli form, bullet-shaped, brick-shaped, tadpole-shaped etc. (Figure 1).

Chemical constitution is simple and in the very small viruses consists of a nucleic acid and protein (Figure 2). The genetic information is contained in the nucleic acid and is centrally located. The central nucleic acid is surrounded by a protein coat called the **capsid**. The capsid is not a smooth structure. It is composed of many sub units called the **capsomeres**. It is the arrangement of the capsomeres that gives the external form to the specific virus particle.

Each virus typically consists of a single type of nucleic acid either RNA or DNA. The presence of either RNA or DNA and never both, is an important property which distinguishes viruses from organisms.

Certain larger viruses may contain lipids, glycoproteins, enzymes and polyamines.

3. Viruses in Action

Viruses are known to attack every kind of living organisms in both the animal and plant world. So far as is known, all the higher plants are susceptible to virus infection and plant virus diseases are the cause of much loss in food production and cause great damage to horticultural plants. The number of viruses attacking plants is about 600 and new ones are still being discovered. Some diseases previously ascribed to viruses are now thought to be due to Mycoplasma-like organisms, viroids, chlamydiae and rickettsiae. Virus have been also found to attack bacteria, mycoplasma, algae and fungi. A virus does not attack any living cell which it comes across. It is specific to the host which it attacks. Tobacco mosaic virus for example will not infect animals and man but will infect tobacco and some other species of plants only.

What happens to a plant when it is attacked by a virus? The steps in the process include:

- (a) *Entry* of the virus into the cell and to the replication sites within the cell. Viruses are obligate parasites-which means they must get into cells in order to multiply. In this they differ from

pathogenic fungi and bacteria. The problem then is how viruses get into appropriate cells. In most cases they are introduced into plant cells by **vectors** or organisms such as insects, mites, nematodes, fungi; etc. which either feed on or parasitise the plants. The viruses are also introduced via accidental or deliberate wounding. Figure 3 illustrates some vectors.

- (b) *Decoating* - The protein coat of the virus is stripped off and the nucleic acid is exposed.
- (c) *Replication* - Viral nucleic acid is replicated and viral coat protein is synthesised. Once inside the cell the viral nucleic acid becomes the presiding deity, inhibits synthesis of host cell protein and host RNA by some mechanism and directs the synthesis of its own RNA and protein at the expense of the host cell materials. Viruses are thus parasites at the genetic level.
- (d) *Assembly* of the newly formed coat protein and nucleic acid to form intact virus particles.
- (e) *Release* - New virus particles move out of the cell through plasmodesmata and in turn infect other cells. Virus particles are carried to other parts of the plant through phloem in most cases and xylem in a few cases.

When replicated virus particles reach the different parts of the infected plant, abnormalities develop in all parts of infected plants, both externally and internally. It is in the leaves of the plants that the most noticeable symptoms develop. Changes also occur noticeably on the stem, flowers and sometimes fruits.

Among the symptoms are mosaics and mottles of different types which accompany varying amounts of blistering and distortion of infected leaves as seen on tobacco leaves infected with tobacco mosaic virus and papaw leaves infected with papaya ringspot virus. In extreme cases leaves may be almost completely chlorotic as in diseases called yellows diseases. There may be extensive thickening of the infected leaf, dwarfing, epinasty, curling and rolling or a combination of these. Examples of diseases showing these are potato leaf roll disease, tobacco leaf curl disease and rice dwarf disease.

Figure 1: Virus Particle Morphology

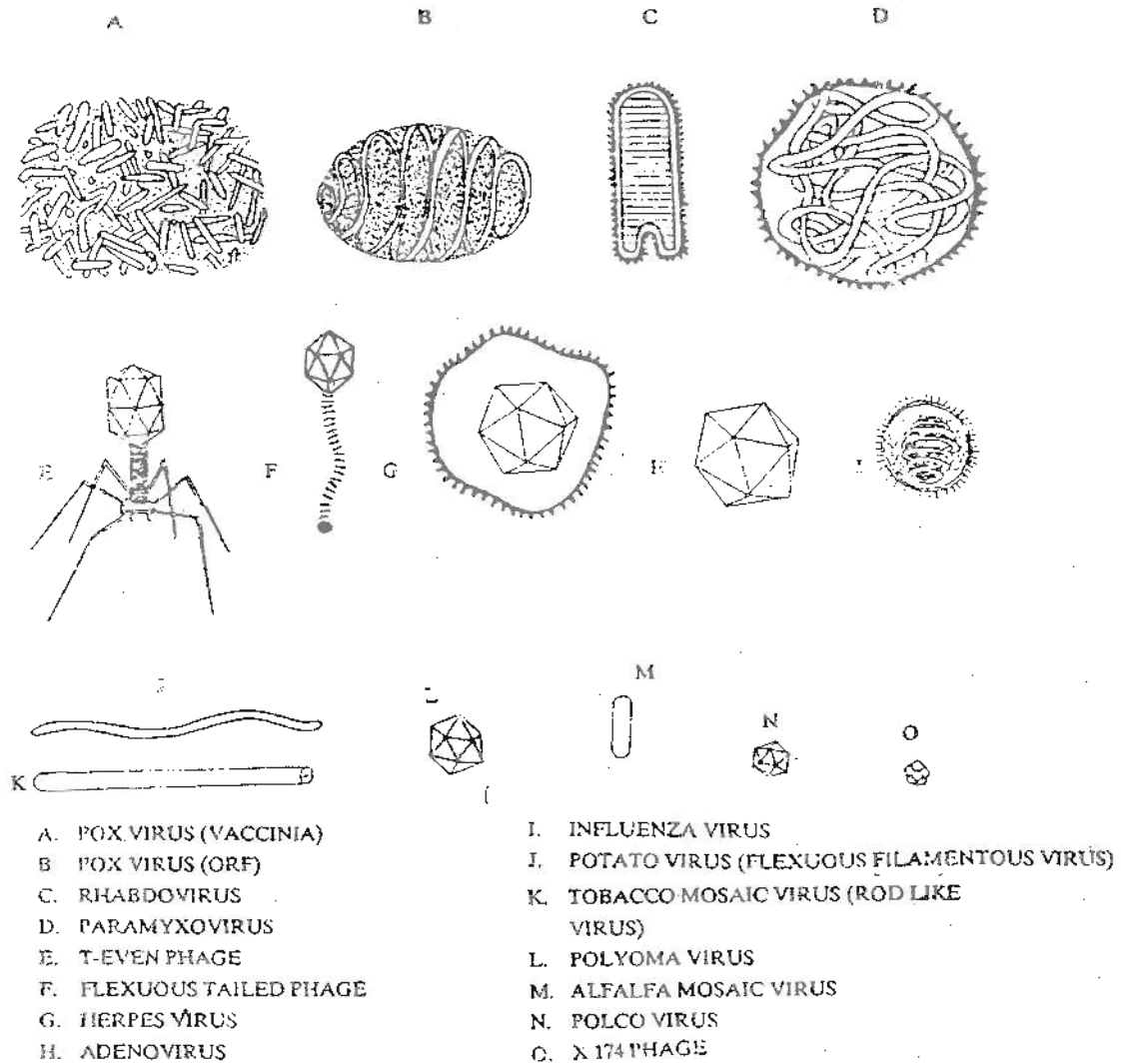
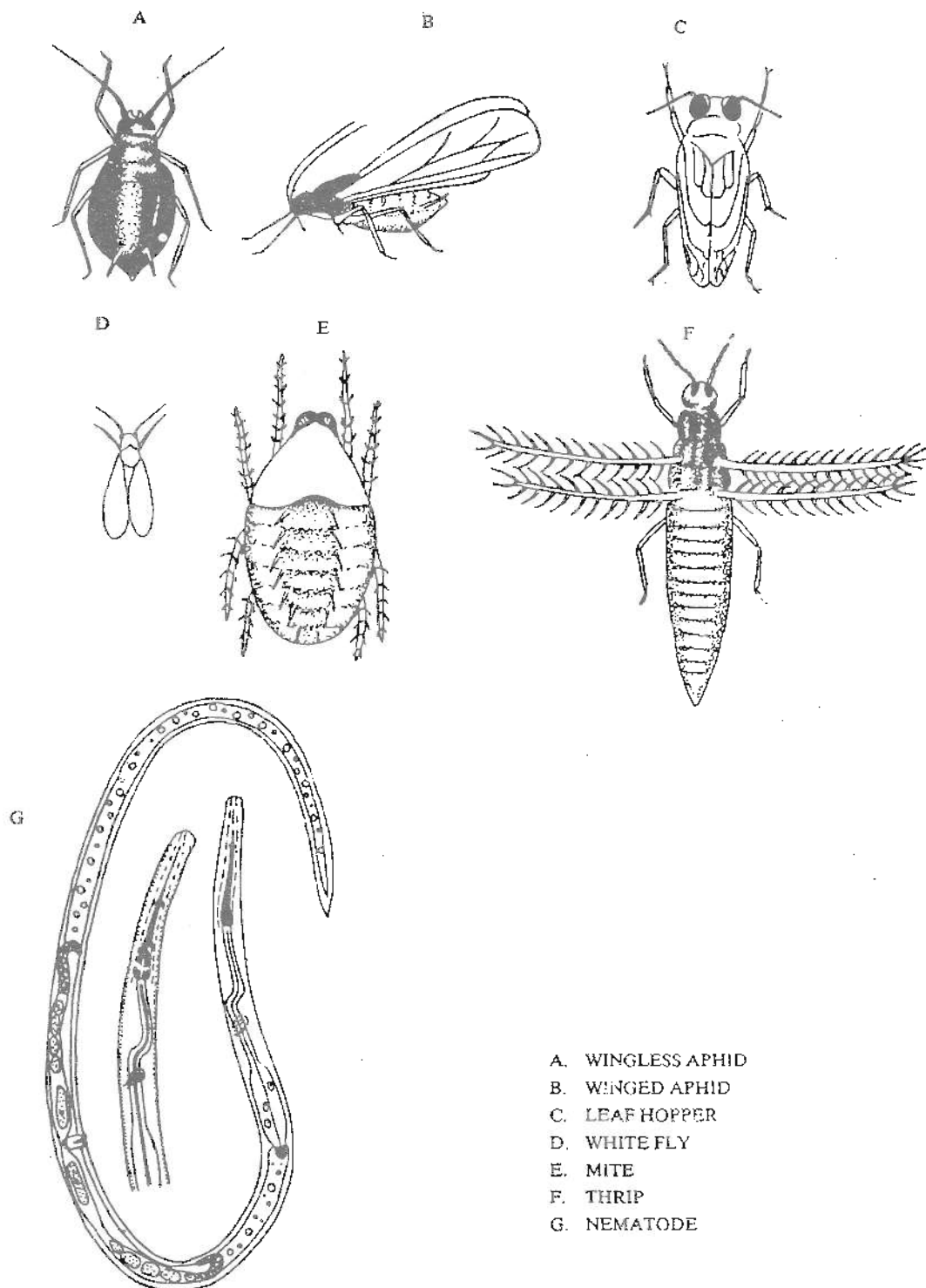


Figure 2 : Structure of Tobacco Mosaic Virus Particle



Figure 3 : Vectors of Plant Viruses



Orugrowths develop on some infections and there may be vein thickenings, vein banding and vein clearing as seen in bandakka yellow vein mosaic. Leaves are sometimes reduced, lamina is greatly suppressed to appear fern-like or shoestring-like as seen to tomato mosaic. Changes in the colour of the flowers are common. Fruits may be affected in various ways such as they may be small, misshapen and bumpy. Some fruits show mottling. It will be apparent from this account that viruses exert a generalised disturbing effect on the metabolism of the infected plant. This in turn will affect the quality, and quantity of the produce of crops.

4. How do Plant Viruses Spread from Plant to Plant?

Plant viruses are more dependent on an agent to transfer them from a diseased plant to a susceptible healthy one than any other plant pathogen.

1. Any factor inflicting wounds on healthy tissue will facilitate mechanical transmission of plant viruses. For example rubbing of leaves against each other by wind in the field is sufficient to transmit potato viruses and tobacco mosaic virus. Viruses like tobacco mosaic virus can be carried on the hands and implements of workers tending the plants in the field. Cutting, pruning or tapping knives also spread infection as in the case of papaya viruses.
2. Some viruses are transmitted by their infected seeds, eg., cowpea mosaic virus.
3. A very important method of spread is by vegetative propagation. If a plant is systemically infected with a virus, all the vegetative parts used to propagate it will contain the virus so that new plants produced by this method will also be virus infected. This method of spread is important in vegetatively propagated plants like fruit trees, potato, roses and sugarcane.
4. All viruses systemically distributed in plants can be transmitted to susceptible plants by grafting. Grafting plays an important role in the spread of fruit and ornamental trees as also in the transmission of citrus diseases.

5. Most important method of spread is by other organisms or vectors. Almost all types of organisms feeding upon or parasitising plants are capable of acting as vectors. A large number of viruses depend exclusively or largely on insect vectors for their transmission. These include aphids, leafhoppers, beetles, whiteflies, mealy bugs and mites. Nematodes and fungi also carry viruses from plant to plant.

Egs. Potato leaf roll virus - aphid
Tobacco leaf curl virus - whitefly
Rice dwarf virus - leafhopper
Tobacco ring spot virus - nematode
Tobacco necrosis virus - fungi

5. Methods of Control

There are various methods of approach to the problem of control of plant virus diseases. All the methods cannot be applied in the same way to all viruses. They would require a combination of two or more methods.

(a) Using virus free planting material

It is of paramount importance that the planting material must be virus free, since many viruses are transmitted by infected seeds and by infected vegetative propagules. If uninfected planting material is not available, it can be obtained either by tissue culture methods or by treating the infected parts with heat in the form of hot water or hot air.

(b) Removal of sources of infection

All sources of viruses such as diseased plants in the field, infected weeds, remnants of previous diseased crops should be removed and burnt. Some viruses, notably tobacco mosaic virus, are contagious to varying extents. Precautions must be taken through washing of hands of workers with soap and sterilising implements by heat or chemicals.

(c) Control by using resistant varieties

This is one of the most convenient methods of control. Virus-immune or resistant varieties are

continuously being bred by plant breeders. Some successes have already been achieved in a few viruses like sugar cane mosaic.

(d) Control of vectors

Some virus diseases can be successfully controlled by means of insecticides against insect vectors. Transmission by vectors can also be controlled by avoiding vectors. Growing of seed potatoes in Pidurutalagala is a practical illustration of a large scale control of potato virus diseases by avoiding the aphid vector. The climate is too cool and moist for the vector. Avoiding the vector can also be done by warding them off the crop by means of screens or cages.

(e) Cure of virus infected plants

In some virus diseases, viruses can be eliminated from infected plants by exposing the infected plants to hot water or hot air for appropriate periods of time. At the moment there is no well established example of practical control of a plant virus disease by means of chemicals acting on the virus in the host.

6. Conclusion

Control of plant viral diseases and the nature of the pathogen are growing fields of study. The more we understand about the pathogen, the agents which spread them, including man and the plant-virus relationships, the better equipped we will be in protecting our crops.

USE OF CHEMICALS FOR PLANT PROTECTION

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Introduction

Sri Lanka's population has grown from an estimated 890,000 in 1824 and 2.4 million in 1871 to a projected mid-year figure of 17.16 million in 1990. It is estimated that the population may eventually stabilise at around 26 million. The present level of available technology requires that the production of increasing amounts of food to feed the growing population necessitates the use of chemicals; although attempts are being made to encourage the use of biological and other non-chemical methods in Integrated Pest Management (IPM). The present account discusses some aspects of the use of chemicals for plant protection in agriculture and forestry, while excluding fertilizers and pheromones (and chemicals used for public health purposes and in households); but including plant growth hormones and wood preservatives registered under the Control of Pesticides Act No. 33.

Uses of Chemicals

Chemicals used as pesticides and for related purposes may be placed in one or more of various categories, such as acaricides, algicides, biocides, fungicides, herbicides (weedicides), insecticides (including, for example, termiticides), insect growth regulators, molluscicides, nematocides, plant stimulants, plant growth regulators, rodenticides, surfactants and wood preservatives.

Each of the above categories may be subdivided again according to their mode of administration or action. For instance, insecticides may be stomach poisons (which enter the pest by ingestion), contact poisons (which enter after the pest touches a treated surface), fumigants (which enter by way of the respiratory system), systemic poisons (which may, for instance, be absorbed through the roots and be transported through the plant to a part on which the pest feeds) and physical agents, such as dusts, which may cause

suffocation, or oils, which damage a protective cuticle. Some chemicals, such as chlorfluazuron, may be Insect Growth Regulators.

Other groups of plant protection products may be composed of chemicals with other types of activities. The Control of Pesticides Act No. 33, also covers chemicals, such as 1-naphthylacetic acid and butralin, which are used as Plant Growth Regulators, and ethephon, which is used as a Plant Stimulant.

Chemical Groups

The different chemical groups differ widely in respect of their modes of action, toxicity, persistence in the environment etc. Some of the commoner groups are as follows:

- a) *Organochlorines* : This group includes many well known pesticides, such as DDT, aldrin, chlordane, dieldrin and heptachlor. Organochlorines and their degraded products may accumulate in the fatty tissues of non-target animals (including human).
- b) *Organophosphates* : This group contains the largest number of insecticides. They exhibit a wide range in characteristics, such as toxicity and persistence. For instance, parathion and disulfoton are extremely toxic and diazinon is relatively persistent. Organophosphates are nerve poisons and, unlike organochlorines, usually do not accumulate in the fatty tissue but are fairly rapidly excreted in the urine.
- c) *Carbamates* : These (esters of carbamic acid) bear some resemblance to organophosphates in that they inhibit the cholinesterase enzymes of the central nervous system. They, also, are not stored in the fatty tissues but are usually readily metabolised and excreted. Chemicals, such as aldicarb and carbofuran,

are systemic insecticides with a long residual activity in the soil.

d) *Pyrethroids* : These resemble natural pyrethrins and have marked insecticidal activity but are, usually, not very toxic to mammals. Pyrethroids, such as bioallethrin, which have a high volatility are useful against storage pests.

e) *Dithiocarbamates and related chemicals* : These (e.g. thiram disulphides) are used as agricultural fungicides and for seed preservation and have low acute toxicity to animals.

f) *Dinitrophenols and pentachlorophenols* : These often have several uses, including wood preservation, but are extremely toxic to animals if taken orally.

g) *Phenoxyacetic acid* : These are used against broad-leaf weeds (for instance, in paddy fields) and include 2,4-D, 2,4,5-T, MCPA and MCPB.

h) *Substituted ureas* : Bromacil and other substituted ureas are used as herbicides, which are fairly non-toxic.

i) *Triazines* : Atrazine, prometryn and terbutryn are members of this group of selective herbicides.

j) *Dipyridylliums* : This group comprises diquat and paraquat, which are herbicides. They are very toxic to mammals if taken orally.

k) *Guanidines and naphthoquinones* : These fungicides include dodine and dichlone.

l) *Trichlorobenzoic, trichloroacetic and trichloropicolinic acids* : TBA, TCA, picloram and other members of this group are herbicides, which are persistent in the soil.

m) *Mercury compounds* : Seeds are, sometimes, treated with mercury compounds to protect them from fungal attack. However, mercury compounds are highly toxic to mammals and are, also, accumulated in the food chain. They should be used with care.

n) *Arsenicals* : Arsenicals, such as methylarsonic acid, include contact herbicides for the control of grass weeds.

o) *Plant insecticides* : These "natural products" include nicotine, pyrethrins and rotenone. While the toxicity of pyrethrins to animals is very low, that of nicotine sulphate is high.

p) *Anticoagulant rodenticides* : These "rat poisons" of the coumarin-type or the 1,3-indandione type inhibit the cessation of internal bleeding; thus leading to death.

q) *Acute rodenticides* : These may be inorganic (e.g. arsenic trioxide and zinc phosphide) or botanical (e.g. red squill) and can be very toxic.

Formulations

While the preceding refers to the active ingredients, which have pesticidal properties, these chemicals have to be "formulated" into preparations that can be used in the field. While there are several reasons why "formulation" is necessary, two of the most important are the possible extreme toxicity of the undiluted ingredient and the need to spread it uniformly over a large area. "Formulations" may, also, contain constituents, which render the active ingredient more soluble or more effective by reducing the rate at which it is degraded within the pest.

When discussing formulations, the term "technical material" is used instead of the name of the chemical or "active ingredient", partly since the purity of the chemical used in pesticide formulations is, for reasons of economy, generally less than that required for laboratory analytical reagents.

The different types of formulations include the following:

a) Dry formulations

1. Dusts - The technical material is mixed intimately with a solid diluent such as finely powdered talc or clay. Plants are treated directly with the dusts. This operation may, however, result in the dust settling in the surrounding areas, as well.
2. Granules - The technical material may be mixed with larger grains of clay or sand and broadcast. A drawback with these formulations is that the

grains may be consumed by birds and other animals.

3. Wettable powders - The technical material is included in amounts of 15 to 95 percent in small, dry particles, which can be made into suspensions with water. The suspensions are then sprayed, as desired, onto the foliage.
- b) Liquid formulations
 1. Emulsifiable concentrates - These concentrates contain technical material, organic solvent and emulsifier together with other products (spreaders and stickers), which enable more effective application of the formulation.
 2. Flowable formulations - These contain a suspension in liquid of fine particles of the pesticide.
- c) Seed treatments - Preparations similar to "wettable powders" are used to protect seeds intended for use as planting materials. Care needs, however, to be taken to minimize the hazards these may pose to birds etc.
- d) Plant drenches - The roots of young plants or rooted cuttings are immersed in a liquid containing a systemic pesticide to allow the latter to permeate the tissues before planting. This is often an effective and environmentally less hazardous means of plant protection.
- e) Poisonous bait - These formulations, where the pesticides are mixed with food, are regularly used for rodent and bird control. Care needs to be taken in their use.

Residues in Foods

Safe practices should be followed to minimise the presence of hazardous levels of pesticides in our daily diet. These practices may include the banning of the import and use of certain pesticides and, for instance, specifying a minimum period of time, which must elapse between the last application of a pesticide and harvesting the crop. It may not be possible to achieve a zero presence of a pesticide in food. However, a legal limit or "maximum residue limit or tolerance "

may be specified and "acceptable daily intake" determined, based on all available information.

Hazard Classification

The hazard or extent of toxicity of a pesticide to an animal depends on a number of factors additional to its chemical nature. These may include the species of animal under consideration, whether the chemical enters through the mouth, skin or lungs and whether the animal has (sometimes unknowingly) previously developed any resistance to the pesticide. The age, sex and diet of the animal are among the other factors, which may have an influence.

A commonly used index to assist the classification of the toxicity of a pesticide is the LD₅₀ value. This stands for Lethal Dose 50 or the dose required to cause the death of 50 per cent of a group of test animals under a given set of investigative conditions. The conditions may include the animal species (such as rats, mice, rabbits or dogs), route of administration of the pesticide (oral, dermal or respiratory), dosage (milligrams of active ingredient per kilogram body weight of the test animal; usually given in a single dose). Many other factors, however, also need to be considered, when assessing the hazards associated with the use of a pesticide. These could, for instance, include long term effects and chronic exposure.

The World Health Organisation has recommended the hazard classification of pesticides as Hazard Class IA (Extremely hazardous), Class IB (Highly hazardous), Class II (Moderately hazardous) and Class III (Slightly hazardous). As an indication, it may be noted that, as regards oral administration of liquid formulations of pesticides to rats, Class IA corresponds to an LD₅₀ of 20 mg or less per kg body weight, Class IB to 20 to 200 mg/kg, Class II to 200 to 2000 mg/kg and Class III to over 2000 mg/kg.

Resistance to Pesticides

Irrespective of the toxicity of the pesticide, its incorrect use can result in pest populations building up a resistance or "tolerance" to it. Individuals may build up resistance during their lifetime (e.g. by increasing the amount of the cytochrome P-450-dependent liver enzymes, which metabolise pesticides) or the increasing resistance may be acquired genetically over

generations. An example is the immunity to DDT acquired by the malaria mosquito in Sri Lanka and the worry that this insect may develop a resistance to malathion if the latter pesticide continues to be used (illegally) for the protection of crops. (Malathion is reserved solely for anti-malaria work in Sri Lanka for this reason).

Resistance acquired by an animal to one pesticide may confer "cross-resistance" to another product, while the "bedding" in cages in which laboratory animals are housed may, also, influence the sensitivity of the animals to the chemicals being investigated.

Use of Pesticides in Sri Lanka

The use of pesticides in Sri Lanka comes under the 1980 Control of Pesticides Act No. 33 and relevant regulations. This Act is implemented by the Registrar of Pesticides, who comes under the Ministry of Agricultural Research and Development. A provisional permit (valid for one year) or a permanent licence (valid for three years) may be granted for a pesticide as recommended by the Department of Agriculture. Labelling and other information to be provided regarding the pesticide is specified and must be provided in Sinhala, Tamil and English.

The pesticides permitted, banned and under other forms of restriction in Sri Lanka are under regular review and the lists are revised, as necessary. As an indication, it may be noted that in 1987 registered pesticides included 405 products and 94 ingredients, including 50 insecticides, 17 herbicides and 27 fungicides. Chemicals, which are banned today, include

2,4,5-T, captafol, chlordimeform, DBCP, dichloropropene (a component of DD soil fumigant), DDT, EDB, endrin, heptachlor, leptofos, parathion, methyl parathion, PCNB (quintozene), thallium sulphate, arsenates and arsenites (prohibited for use as weedicides in agriculture but sodium salts are used in wood preservatives) and mercury compounds (prohibited for use in agriculture even as a seed dressing or as a panel dressing. However, organomercurial biocides are used in the paint industry).

Finally, it should be remembered that a pesticide may be lethal to honey bees and other insects, which are valuable for pollinating certain flowers. For this reason, insecticides are, sometimes, sprayed in the late evening in order to reduce the exposure of beneficial insects, as much as possible.

Acknowledgements

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Further Information

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GUARDIANS OF THE PADDY CROP

MANOHARI SANDANAYAKE

Scientific Officer

Natural Resources, Energy and Science Authority of Sri Lanka

Rice is our staple diet and paddy occupies the most important place among our food crops.

In order to feed the growing population, every possible effort has been made to increase the output of paddy through expansion of cultivation and by improving the yield.

However, the achievement of these objectives has been seriously affected by several factors, and insect pests remain the most formidable threat of all.

On a conservative basis, it has been estimated that insect pests account for as much as 20 - 30% of the loss in the potential yield of paddy.

Application of chemical insecticides has been the major means used in the control of insect pests, but even after a sustained usage over a long period of time, these chemical agents have not been able to eradicate or even bring down to minimal levels - the threat posed by insects.

Although, from a short term perspective, a certain degree of success has been achieved through chemical agents, the time is now ripe for a realistic long term assessment of the advantages and disadvantages of chemical insecticides.

The major drawback inherent in chemical insecticides is related to the problem of toxic chemical residues that remain in the crop and eventually enter and accumulate in the bodies of human beings. The second aspect is the ability of insects to develop resistance to many insecticides. Once a particular insect species develops resistance to a specific insecticide, invariably it requires a more toxic agent to bring them under control.

The development of such insecticides requires time, effort and money and the new insecticides tend to be expensive and beyond the means of ordinary farmers.

Usually, a range of insect species are found in any paddy field. The majority of these are really protectors of the paddy crop. The cultivator, who cannot distinguish between the useful and harmful insects resorts to indiscriminate application of insecticides, thereby inadvertently laying the foundation for a more sinister long term threat.

Therefore, the ability to distinguish between these two types of insects is an absolute necessity in paddy cultivation.

Insect Pests

First, let us look at the pests. The major insect pests of paddy (in the dry zone as well as in the wet zone) are;

1. Yellow Stem borer {*Tryporyza incertus* (Walker)}
2. Leaf folder {*Cnaphalocrocis medinalis* (Guenée)}
3. Paddy gall midge {*Orseolia oryzae* (Wood-Mason)}
4. Brown plant hopper {*Nilaparvata lugens* (Stål)}
5. Rice leafhopper :
 - 5a. {*Nephotettix virescens* (Distant)}
 - 5b. {*Nephotettix nigropictus* (Stål)}
 - 5c. {*Nephotettix impicticeps* (Ishihara)}
 - 5d. {*Tettigella spectra* (Distant)}
 - 5e. {*Inazuma dorsalis* (Motschulsky)}
6. Rice thrip {*Baliothrips bifurcata* (Bagn.)}
7. Paddy bug {*Leptocoris varicornis* (Fabricius)}

In addition to them, there are also several insect species which can be termed "minor pests". These are : Stem fly, Whorl maggot, Case bearer, Leaf eating larvae and the Paddy bug.

The two insects, yellow Stem Borer and Leaf Folder (1 and 2) damage the paddy plant in their larval stage. The female insect lays eggs on the paddy leaves. The larvae that come out of these eggs either bore through the stem or make nests by folding and sticking the paddy leaf. Thereafter, they feed on the plant sap,

leading to withering and eventual death of the plant. The other insects usually attack paddy plants in their adult stage. They feed on the plant sap from stems, leaves or even suck the paddy kernel while it is in the liquid stage. Dead and dying plants with withered buds and whitish leaves as well as empty seeds (white in colour) are the indications of the presence of these pests in paddy.

Let us now look at the predators and parasites of these pests.

Predators of insect Pests

The colourful Lady Bird Beetle (Figure 1) is a small insect which spends all stages of its life cycle in paddy. From the time it is hatched out of the egg it feeds on the eggs, larve, pupae and adults of all other pests.

The Cricket shown in Figure 2 is adept at jumping from plant to plant. In common with most insects that inhabit paddy, the female Cricket lays her eggs on the leaves of paddy plants. Like the Lady Bird Beetle, the Cricket also feeds on pests in all stages of their life cycle (from the time it is hatched as a larva).

The Grasshopper (Figure 3) is a familiar insect. To a certain extent it feeds on the young paddy shoots but performs a much more valuable service in devouring all insect pests. A similar role is played by the Water Bug (Figure 4).

The Plant Bug shown in Figure 5 is an interesting insect, because it has the ability to catch insects several times bigger. To benumb its prey, the Plant Bug injects a poison into the intended victim. The poison sacs are conveniently located inside its mouth. Prey consists of larva of all insect pests, flies, moths and butterflies.

The Rice Thrip (Figure 6) is another interesting insect. The early stages of its life cycle are spent in water and it comes out to inhabit paddy plants as an adolescent. At this stage, it feeds on the larvae and pupae of paddy pests. The adult Thrip feeds on flying insects such as flies and moths.

One of the most efficient insects, the Paddy Bug, is shown in Figure 7. It is quite capable of locating and

feeding on the pupae of Yellow Stem Borers and Leaf Folders hidden in various parts of the paddy plant. It is estimated that the Paddy Bug devours about 20-30 larvae per day.

Several species of ants are shown in Figure 8. They too perform a valuable service by feeding on all stages of all pests.

Spiders (Figure 9) adopt a different technique for catching paddy pests. They are expert tappers, and the trap is none other than the cob web familiar to all of us.

Parasites

Paddy pests are not invincible beings. Apart from the predators described earlier, they have their own quota of parasites to contend with.

Three such groups of parasites are described below :

Group I

Two members of this group of parasites are shown in figures 10 and 11. They spend their entire life cycle within the eggs of paddy pests.

Group II

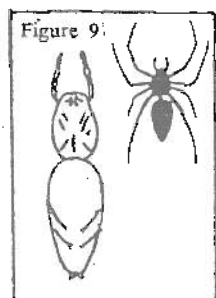
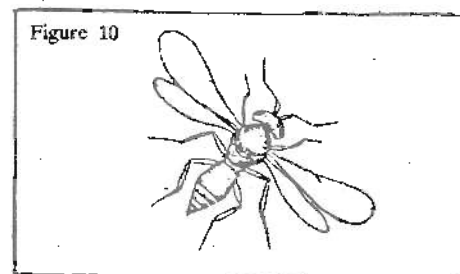
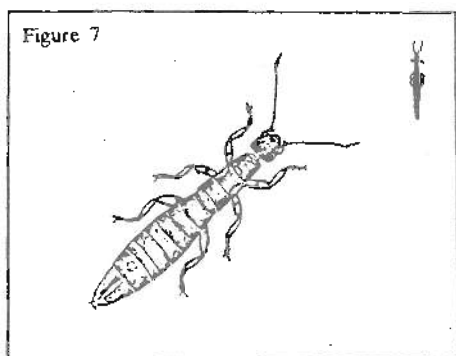
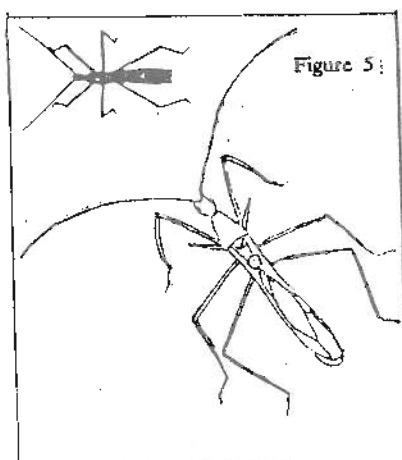
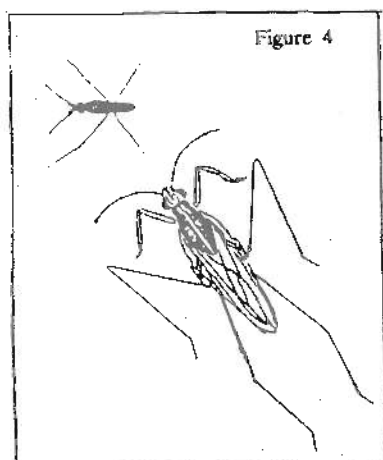
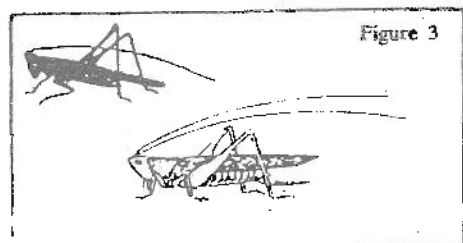
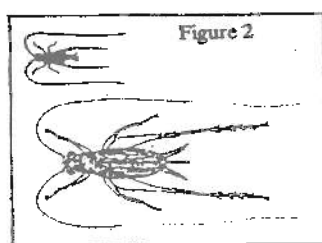
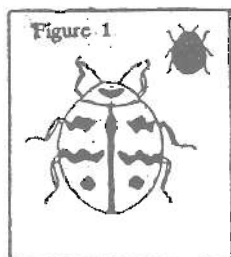
The life cycle of these parasites (of which one is shown in figure 12) is spent in the eggs and the larval stage of insect pests.

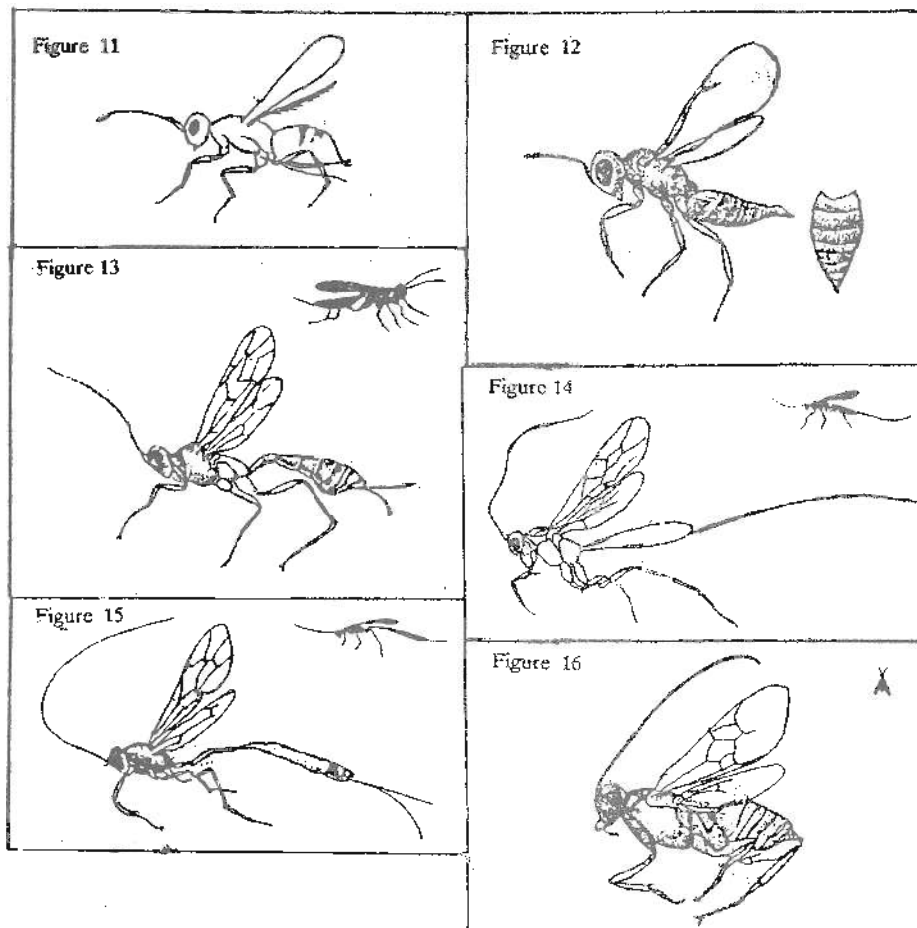
Group III

These probably are the deadliest enemies of paddy pests, because they lay their eggs inside the body of pests. Once the eggs are hatched, the emerging larvae feed on the body of the host, thus ensuring its total destruction. Four such useful parasites are shown in figures 13 - 16.

The facts given above reveal the useful role played by parasites in the control of insect pests in paddy plants.

In terms of destructive power, it may be noted that these parasites pack sufficient punch to knock off superior numbers of their adversaries. For instance, a female parasite lays between 40 - 160 eggs. Each of





these eggs in capable of destroying at least one pest insect and add another one to the parasite population.

The chief distinguishing feature of these parasites is the ovipositor that projects outwards like a tail. Several species (as mentioned earlier) have hidden ovipositors.

The pair of antennae is yet another feature, the function of which is well known.

The predators and parasites of paddy pests are really our first line of defence. It is our duty to recognise and look after those that man the forward defence positions.

AN INTRODUCTION TO THE THEORY AND PRACTICE OF PLANT TISSUE CULTURE

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A major role of modern science and technology is to provide for the increasing requirements of food, medicine, clothing etc. of the expanding human population on earth. Plant tissue culture can contribute to the increased production of food, fibre, medicinal and other useful plants. This technology has been already employed to increase the yield, pest resistance and tolerance to environmental stresses of several agricultural crops. Therefore, an understanding about tissue culture and its potential use is important.

History of Tissue Culture

Before a detailed discussion of tissue culture technology, a brief historical sketch is given here. Gottlieb Haberlandt, a German Botanist is considered as the father of Tissue culture. He was the first person to grow parts of plants in an artificial culture medium. These experiments conducted in 1898 involved several plant species. For example, he used cells from the palisade parenchyma of the species *Eichhornia crassipes* and *Labium puerperium* and also the leaf epidermal cells of *Oenothera lamarckiana* sp. for artificial culture. The culture medium employed here was Knop's salt solution enriched with sucrose.

After several days of culture in this solution, Haberlandt observed a significant growth in size of the palisade cells. Although, there was no cell division in this medium, Haberlandt could describe the method of culture of cells isolated from a parent plant in an artificial medium. He could also propose several hypotheses in this field of science which were proved to be correct by the subsequent work of other scientists. The concept of growth control substances proposed by Haberlandt is one of his major contributions. He named these substances as growth enzymes and he believed that they are produced by a specialized

group of cells. The secretions of these cells were believed to affect the growth of other cells.

Since the work of Haberlandt, many other scientists have contributed to improve the plant tissue culture technology to its present advanced status. Among them, the contributions of Philip White, Morel, Skoog, Murashige, Miller, De Fossard etc. are worth of special mention. These and other scientists identified the factors that are responsible for the failures at different stages of tissue culture and found methods of overcoming them. Thanks to these findings, plant tissue culture has attained so much success that it is now being applied in a multitude of food, ornamental and medicinal plants for their improvement.

Physiological basis in tissue culture

Cell totipotency

Even a huge tree has been formed from a single cell called the zygote which is capable of division and subdivision giving rise to an intact plant. This ability of a cell is called totipotency. *In vitro* culture techniques are based on this concept and according to that appropriate conditions should be provided for the initiation and regulation of the cell division and subsequent cell differentiation and regeneration of plantlets (small plants).

In tissue culture procedures, usually a normal differentiated tissue with non dividing, quiescent cells are plated on a culture medium. These cells first undergo certain physiological changes due to interaction with the exogenous growth regulators. As a result of this they acquire a meristematic state. This phenomenon is called dedifferentiation and usually results in the callus formation. In further interaction with some factors in the culture medium those dedifferentiated cells in a callus tissue could again become capable of

forming an intact plant or plant organs. This phenomenon is termed **redifferentiation**. Thus, for a non-dividing, mature, differentiated cell to express its totipotency it should become dedifferentiated and only then to be redifferentiated. On the other hand the redifferentiation can take place directly from dedifferentiated cells avoiding the callus formation.

Basic Requirements for Plant Tissue Culture

Tissue culture is the growing of a part of a tissue of a plant (or an animal) or a part of it in a medium containing nutrients under aseptic conditions in a closed environment (*in vitro*). For example, a part of the terminal bud of a plant can be made to grow in a medium containing all necessary nutrients solidified using agar. This medium should be contained in a culture tube or a flask. Moreover, the explant (part of the plant used for tissue culture), the medium etc. should be free from micro-organisms and therefore should be disinfected and raised under closed conditions.

Culture medium

For cell division and subsequent differentiation, a plant cell *in vivo* requires numerous nutrients, growth regulators etc. Similarly an artificial medium (culture medium) should also provide all those vital constituents to carry out the normal cell functions *in vitro*. Usually a culture medium consists mainly of macro elements (elements required in greater concentrations than 0.5m. mol per liter), micro elements (elements required in lesser amounts than 0.5m. mol per liter), vitamins, plant hormones, sugar etc.

Macro and micro elements

Besides carbon, hydrogen and oxygen there are about 12 elements required for normal plant growth. They are nitrogen, phosphorus, sulphur, calcium, potassium, magnesium, iron, manganese, copper, zinc, boron and molybdenum. Among these elements the first six are macro elements while the rest is considered as micro elements. Both macro and micro elements should be added in proper concentration recommended for particular medium and lack of elements may affect the *in vitro* plants.

Carbon source

Carbon can be considered as the most important element in the organic processes. Therefore, it is essential to provide a carbon source to the culture medium. Sucrose, glucose, fructose are used for this purpose and among them sucrose is the most preferred. Sugars such as maltose, galactose and lactose are rarely utilized.

Vitamins

Vitamins also play a major role in many biological processes of *in vitro* cultures as well as *in vivo* plants. Some vitamins such as vitamin B₁ (Thiamin), vitamin B₆ (Pyridoxin), vitamin B₂ (Riboflavin), vitamin C (Ascorbic acid), vitamin B with vitamin M (Folic acid), vitamin H (Biotin) and Nicotinic acid are the more important in tissue culture. Moreover, a carbohydrate called myoinositol (Inositol) is also used as a constituent of vitamin B complex. Vitamins function as coenzymes (as a part of enzymes) and take part in various important reactions of plant metabolism. However, it is advisable to use the vitamins in appropriate concentrations recommended for the particular medium.

Plant hormones

The objective of plant cell, tissue or organ culture may be to obtain either a new form of a plant or a callus. A callus is a mass of unorganized cells formed as a result of division of a cell/cells. By manipulating tissue culture media and conditions it is possible to regulate the development path-way of cells of *in vitro* cultures (see fig. 1).

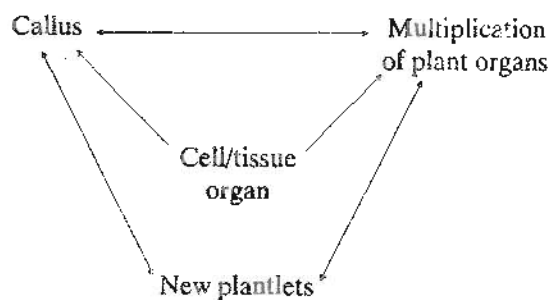


Figure 1: Several stages of plant tissue culture and their inter-relationships

There are five main groups of plant hormones viz auxins, cytokinins, gibberellins, abscisic acid and ethylene. Among these hormones only auxins and cytokinins are frequently used in tissue culture media. Gibberellin and abscisic acid are rarely used. Usually the ratio of the concentrations of auxins and cytokinins used in culture media determine whether the cultures would give rise to roots, shoots or callus tissues.

Auxins

In 1926 F.W. Went Coined the term auxin which was derived from a Greek word *auxin* (to increase). In nature, plants contain auxins in very small quantities and it is synthesized in meristem tissues of shoots and root-tips, developing leaves, flowers and fruits. This group of hormones is involved in the regulation of *in vivo* processes like elongation of stem and internodes, tropisms, apical dominance, abscission, rooting etc. In tissue culture auxins are used to induce cell division (for example to obtain callus tissues) and root differentiation. There are several auxins like indole-3-acetic acid (3-IAA), 3-indolebutyric acid (3-IBA), α -naphthalene acetic acid (α -NAA), 2,4-dichlorophenoxy acetic acid (2,4-D) and 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) which are used in the preparation of culture media.

Each member of this group behaves in a unique manner in culture media *in vitro* and the effect of different hormone on the same tissue under similar conditions may be different.

For example, 3-IBA and α -NAA are useful in inducing root differentiation of cuttings while 2,4-D and 2,4,5-T are very effective for the induction and growth of callus. Moreover, 3-IBA and α -NAA in interaction with cytokinins can be used for shoot proliferation.

Cytokinins

The word cytokinin in latin means cell division. In nature, cytokinins are found in different tissues of plants such as root-tips, ripening fruits, germinating seeds, xylem sap etc. and are important in such processes like cell division, modification, apical dominance and shoot differentiation. In tissue culture, cytokinins are used for cell division and differentiation of adventitious shoots from callus and organs. Among fre-

quently used cytokinins 6-benzylaminopurine (6-BAP), 2-isopentenyl adenine (2ip) and kinetin can be considered as the most effective.

Other growth stimulants

While vital constituents such as macro and micro elements, vitamins and hormones are essential in the medium, other substance such as potato extract, yeast extract and coconut milk (coconut water) may enhance the growth processes on *in vitro* cultures. However, they should be added only if necessary as their composition can vary from one sample to another.

Agar as a gelling agent

In tissue culture, nutrient media can be in liquid state as well as in the solidified form depending on the purpose of the culture. These two states of media have their own advantages and disadvantages. For example, in liquid media, tissues are submerged and this could lead to the death of tissues due to lack of oxygen, unless proper measures for aeration are taken.

Therefore, sometimes media have to be solidified with a gelling agent like agar, a polysaccharide obtained from the extracts of seaweeds (a red algae). Agar promotes gas exchange and the diffusion of nutrients. The optimal concentration of agar is about 0.8%. Besides agar, there is another gelling agent called Gelrite available commercially. It is produced from bacteria (*Pseudomonas* spp.).

pH value of the medium

The pH value of the medium is an important factor and therefore its maintenance on the optimum is very essential. For example, pH value affects the gel strength of the medium. Usually the lower the pH value the softer is the medium. With the increase of pH the hardness of the medium will increase. Therefore, deviation from the optimum pH value could be harmful to the plant material.

There are numerous tissue culture media used for different culture purposes. Among these, media of Murashige and Skoog, (MS), N₆, Gamborg (B₅), Eeuwens (Y₃), White and Blaydes are widely used. For example, we have used three media namely MS, N₆ and Blaydes for rice anther culture experiments and N₆

was the most suitable medium for many varieties. Potato extract enhanced the callus induction of those varieties.

Explant

Tissue culture techniques are being used in several specified sections whose names have been formed according to the explant, a plant part or part of the organ used to initiate the culture. Due to numerous applications of tissue culture many parts of plants have been used as the initial material and based on these parts (explants) different sections of tissue culture, can be now identified (see table 1).

Table 1: Main sections in plant tissue culture

Nomenclature	Examples
1. Leaf culture	Anthurium, Coffee, Cauliflower
2. Embryo culture	Coconut, Carrot, Almond
3. Shoot-tip culture	Onion, Cabbage, Potato, Sweet potato, Cassava (Manihot)
4. Root-tip culture	Oil palm, Cauliflower
5. Anther culture	Rice, Tobacco, Barley
6. Cell culture	Tomato, Potato
7. Axillary bud culture	Pineapple, Ginger, Sweet potato Cucumber
8. Protoplast culture	Tomato, Carrot, Tobacco

Microbes like bacteria and fungi are capable of fast growth in culture media and thus form metabolic wastes which may be toxic to plant tissues. Therefore, almost everything used in tissue culture including culture vessels, culture media, plant material, equipment, culture - transferring area etc. should be sterilized before the culture procedure.

Laminar air flow (LAF) cabinet allows a sterile and particle free (microbe-free) environment, providing a micro-filtered ultraclean air flow in which the tissue culturist can perform the culture procedure. If a LAF cabinet is not available, a special room may be modified for the culture operations. An ultra-violet (UV) sterilizing lamp promotes to avoid microbial contaminations. But UV lamp should be handled carefully as its rays can be harmful for skin.

Large number of microorganisms are found on the surfaces of plant tissues. Therefore, surfaces of plant tissues should be sterilized thoroughly before culture. There are several sterilizing agents used to decontaminate plant tissues. Calcium hypochlorite, Sodium hypochlorite, Bromine water, Hydrogen peroxide etc. are successfully used in many tissue culture procedures. However, the sterilizing agent to be used should not be toxic to the given plant tissues. Optimal concentration of sterilants and the adequate duration of their treatment should be chosen for an effective sterilization.

All instruments used in tissue culture technology like forceps, knives, blades etc. should be dipped in 96% ethyl alcohol and sterilized in a spirit lamp-flame.

Autoclave serves to sterilize media vessels and other required items by steam under a certain pressure and temperature. However, a household pressure cooker can also be for the same purpose if an autoclave is not available. A great care should be taken not to exceed the required duration of autoclaving since it affects the culture medium, destroying some vital components in the medium.

Culture room

A culture room should provide appropriate and adequate conditions to store cultures under optimal conditions (i.e. it should provide optimal temperature, light intensity, photo period and if possible humidity) / and to observe them. It should possess a microbe-free environment at least to a certain extent. Most culture rooms are equipped with fluorescent lamps, air conditioned environment with humidities.

Transplanting of healthy materials into soil (Hardening)

Since *in vitro* plantlets are grown on culture media under special conditions their anatomical, morphological and physiological characteristics are different from those grown under natural conditions. *In vitro* plantlets are grown in a closed space with high relative humidity. Therefore they exhibit several abnormalities in water relations and have reduced photosynthetic capacity. For example, reduced

deposition of cellulose and lignin causes the reduction of cell wall pressure leading to increased water uptake by cells and a glassy turgescence of leaves and stem. Furthermore, development of epicuticular wax on the leaves of *in vitro* plantlets is poor. The stomata are usually characterized by their malfunctioning, failing to close when needed.

All these changes result in the rapid wilting, slow growing and also high rates of mortality of the plantlets upon their transfer to *ex vitro* conditions. These drawbacks should be minimized to improve the survival of *ex vitro* plantlets. Plantlets have to be removed from culture vessels and washed thoroughly and transferred to the soil where microbial organisms are minimized. Usually the plantlets are acclimatized in a humid environment *ex vitro* and transferred to grow under normal greenhouse conditions after a certain period. This procedure is called hardening.

Some practical applications of plant tissue culture

Although for developing countries like Sri Lanka tissue culture technology is still economically not beneficial, it may be a very profitable field in the near future. Nowadays, tissue culture technology is being applied in many areas. Some applications that are currently in use or will be useful in the near future for Sri Lanka are discussed below.

In vitro propagation

By *in vitro* propagation, we mean the propagation of plants in a controlled, artificial environment using culture vessels aseptic techniques and a defined culture medium. For this purpose usually a small part of plants (e.g. -shoots) is cultured. Some explants give rise only to shoots while others can form both shoots and roots. In case of only shoot formation those explants should be transferred to another medium for rooting.

In vitro method can be used to multiply pathogen-free plants by using meristem-tip or shoot-tip explants. This is possible because the growing point of the shoot is free from pathogens even in infected plants. Another advantage of *in vitro* propagation is that the cultures can be stored under low temperatures to stop their growth, when there is no need for planting material. They can be made to grow again when

needed. However, the greatest advantage of *in vitro* propagation is the possibility of multiplying an elite genotype in large numbers to obtain genetically uniform plants.

In vitro Orchid production

The commercial importance of orchids makes it necessary to improve and propagate them in large scale. Presently in Sri Lanka besides pot culture, hush culture, bedding etc. tissue culture methods are also practiced for propagation of orchids. *In vitro* methods for micropropagation consist of four main steps:

1. *In vitro* culture for pathogen-free plant material
2. Shoot multiplication
3. Root formation
4. Hardening

There are many specified media used for orchid propagation. Some of the widely used media are Krudson C orchid medium, Lindemann orchid medium and Vacin and Went medium. Usually shoot-tips are used as explants and they should be cultured aseptically.

Shoot-tips usually form round, glistening structures called protocorms, which may be multiplied indefinitely or induced to regenerate a whole plant. This protocorm mass should be chopped into several pieces and subcultured on a fresh medium to obtain more structures. This procedure can be repeated according to the requirements. Culture of chopped protocorm pieces on a regeneration medium promotes shoot formation. Thus, *in vitro* multiplication of orchids can produce several millions of plants as against a few hundred by conventional propagation techniques.

In vitro embryo culture

Embryo culture is very essential in difficult crossing programmes in plant breeding. For example, embryo rescuer technique has helped to overcome the difficulties in several interspecific crossings of important crops like rice, barley, bean, pepper, papaw, tomato etc. Moreover, embryo culture *ex-ovule* (out side the environment of the ovule) can be used to study the nutrient requirements of the embryo during its development. Seed germination of some species possessing long periods of dormancy (deep dormancy) is

determined by their morphological, physiological and biochemical factors. *In vitro* embryo culture may help to overcome deep dormancy and initiate germination because in most cases the factors influencing the dormancy are located in the seed, outside the embryo. For example embryos of some coconut varieties like "Dikiri pol" and Makapuno do not germinate within its own endosperm. Excised embryo culture promotes their growth *in vitro*. Furthermore, excised immature embryo culture not only bypasses the stage of dormancy but also tends to undergo the embryogenic mode of development.

Haploid plant production

Haploid plants possess a single copy of the chromosomes characteristic for the given species. The success in haploid plant production from anther and pollen grains has helped to obtain homozygous lines within one or two generations in more than 200 species. Since anther culture techniques offer the possibility to obtain genetically diverse haploids, by doubling their chromosomes the plant breeding process can be expedited. Usually colchicine is used for chromosome duplication, although spontaneous diploidization has also been found in some species.

In vitro development of plants from male gametophytes (*in vitro* androgenesis) can be divided into the following.

1. Androgenesis of microspore - Here non-zygotic, embryo like vegetative structures called embryoids are formed in culture which are capable of developing into embryos and subsequently to whole plants. Haploid plants are developed from pollen grains in this case.
2. Androgenesis of callus - Haploid plantlets are derived from callus which is usually formed within an anther.
Example - Rice (*Oryza*), Barley (*Hordeum*)
3. Androgenesis of microspore and callus - Both processes are taking place but callus formation phase is so short making it difficult to study the organogenetical processes without special cytological approaches.

Suspension culture

The culture in which individual cells or aggregates of cells multiply while suspended in liquid medium is called suspension culture. Usually inoculation of friable callus tissues to a liquid medium and subsequent shaking by an orbital shaker can maintain a homogeneous population of cells. If calli are not available, other plant organs can be selected, surfaced-sterilized and used as the first inoculum. Agitation of the medium is very essential since it is capable of breaking cell aggregates into smaller clumps and single cells maintaining and uniform cell distribution in the medium.

Cell suspension cultures are used to study secondary metabolism in cells, enzyme induction and gene expression. They also serve as a source material for enzymes in industry.

In vitro fertilization

This is also called test-tube fertilization. These techniques can be applied to overcome drawbacks like self incompatibility in some species and cross incompatibility appearing in interspecific and intergeneric hybridization. For example, an interspecific hybrid *Nicotiana tabacum* X *Nicotiana glauca* had been produced by using this technique. This method could also be applied for self-pollination of such species which have mechanisms to prevent it. A fertilized zygote can be made to grow in an artificial medium without any rest. Therefore, pre-fertilization of pre-zygotic barriers and embryo-endosperm incompatibility and other barriers can be avoided by this technique. To fertilize the excised plated ovules surface sterilized pollen grains are to be placed.

To perform *in vitro* fertilization surface sterilized pollen grains are to be placed on the excised ovules plated on a suitable medium.

In vitro germ-plasm conservation

Massive and indiscriminate deforestation has led not only to global weather changes resulting in drought and increasing green house effect, but also to the disappearance of some species from the flora. There-

fore, efforts should be taken to conserve the threatened flora. One of the remedies for this problem can be *in vitro* germ-plasm conservation since this technology make a it possible to safeguard the genetic material in a relatively little space in a viable form for future use. This technology also promotes to maintain pathogene-free germplasm collections. *In vitro* germ-plasm conservation can be performed by four basic methods,

1. Maintenance under normal growth
2. Growth limitation
3. Cold storage
4. Cryopreservation (freeze conservation)

Among them the last three approaches are most effective in contrast to the first one.

Selection for stress factor resistances by *in vitro* techniques

Cultured plant cells or tissues are unique objects for pure and applied research and are ideal material to produce varieties with improved disease or stress tolerance. This method is based on spontaneous or induced mutagenesis *in vitro* and after which cells will be selected. Plants are regenerated from showing resistance to the stress factor cells. For example, salt-tolerant varieties of several species like rice, alfalfa etc. have been obtained from callus tissue. Screening for drought-tolerant coconut forms is reported to have been successful by using *in vitro* embryo culture.

Selection for disease resistant varieties by *in vitro* techniques

One of the major objectives in plant breeding is to obtain varieties with improved disease resistance. Tissue culture methods can be used for the screening of disease resistant forms within a relatively short period. The methodological aspects of this technology are almost the same as those of screening for stress tolerance. The production of disease resistant plants by *in vitro* selection involves several steps.

1. Exposing cultures to certain concentrations of phytotoxin or compounds which are similar to phytotoxin produced by the pathogen.
2. subculture of survived cultures.
3. Regeneration of plants from the survived cultures.
4. Test for the resistance of plants to the particular pathogen.
5. Studying the inheritance resistance.

In spite of several drawbacks, *in vitro* technology in selection for disease resistance has many advantages. For example, this method allows to conduct the selection procedure in a relatively small space during a short period compared to conventional methods.

Presently, *in vitro* technology is an expensive methodology for developing countries like Sri Lanka. But, in the near future it will be one of the more effective and economically feasible techniques in plant breeding and propagation. However, a great care should be taken to avoid dangerous consequences that can result from improper handling.

ENERGY IN THE BIOSPHERE

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All living systems need to expend energy not only for growth but also for survival. In living cells there is a constant synthesis of macro molecules from smaller units, and to drive the reactions concerned, a source of energy is required. Where does the energy needed by living organisms come from? The ultimate source of energy for all organisms, save for a few specialist bacteria, is the sun. Solar energy is captured by green plants through the process of photosynthesis, where photon energy is converted into chemical bond energy trapped in organic molecules. A small group of autotrophic bacteria - the chemotrophs - differ from normal green plants in that they do not need solar energy for carbohydrate synthesis, but are able to utilise chemical energy for this purpose. This chemical energy is obtained by oxidation reactions.

Solar energy fixed by green plants drives all the life processes in the biosphere, being transmitted from organism to organism through various food chains, while a part of it is re-radiated into space as heat and light.

The earliest known forms of life are the prokaryotes which evolved about 3.5 billion years ago in the primordial seas. These early organisms were not autotrophs and lived in an atmosphere devoid of oxygen. They probably obtained their energy by a breaking down of organic molecules by a process similar to that of fermentation. The emergence of the autotrophs was a major step in evolution. The prokaryotes gave way to the eukaryotes, and with a further build up of organic matter by early autotrophic eukaryotes the way was clear for the evolution of multicellular metazoa which depended on these autotrophs for food. They obtained their energy by the breakdown of food (produced by early autotrophs) in the presence of oxygen, by a process termed respiration. The oxygen was obtained from what was gradually built up in the atmosphere by the photosynthesis of eukaryotic autotrophs. The autotrophs themselves developed into more and more complex forms during evolution and were finally able to colonise the land.

With the colonisation of the land, over two billion years ago, new forms of fauna and flora developed, and it was then possible to fix more energy and support more life. The green plants (including the algae) are the main organisms capable of trapping and using solar energy. This occurs during the process of photosynthesis. Photosynthesis is one of the most important biological processes since it is the process by which energy from sunlight is harnessed for the fixation of carbon dioxide into organic compounds.

The green plants are autotrophs which are independent of an external supply of organic compounds for their energy requirements. They manufacture organic compounds using solar energy. Of the total radiant energy that falls on earth only about one per cent is trapped by green plants in photosynthesis. But in tropical evergreen forests and grain fields, sometimes, the figure rises to about 4 to 5 per cent. Though the proportion of energy fixed is very small, worldwide, it is equivalent to the annual production of between 150-200 billion tons of organic matter, and it includes both food for man and the energy which runs the life support systems of the biosphere i.e. the major ecosystems. Of the rest of the radiant energy, i.e. 99 per cent, a small fraction is used in the evaporation of water from the large expanses of oceans and lakes, while the rest is re-radiated into outer space as heat and light.

Solar energy is absorbed by the green pigment chlorophyll which is found in green plants (including algae). Chlorophyll in its forms of chlorophyll a and b absorbs the blue and the red of solar rays and becomes activated. The activated chlorophyll is then utilised by the plant to split up water during the light reaction of photosynthesis. The splitting up of water, called photolysis, results in the release of oxygen to the atmosphere, while the hydrogen combines with an organic compound nicotinamide adenine dinucleotide phosphate (NADP) to form a reducing factor NADPH_2 . Energy released during the light reaction is used for the formation of the energy rich compound Adenosine triphosphate (ATP). The reducing factor

NADPH₂ and the energy rich ATP are necessary for the reduction of the first formed carbon compound in photosynthesis.

During the stage of photosynthesis which does not require light (called the dark reaction) carbon dioxide combines with a 5-carbon compound called ribulose diphosphate (RuDP) to give an unstable 6-carbon compound. This unstable 6-carbon compound breaks down to give the 3-carbon compound phosphoglyceric acid (PGA) which is the first stable carbon compound formed in photosynthesis. The PGA is reduced to phosphoglyceraldehyde (PGAL) by the NADPH₂ during the dark reaction and the energy necessary for the reaction is provided by the ATP. The end product is a hexose sugar formed by the combination of two molecules of PGAL. The hexose thus formed has trapped within it part of the energy obtained from the sun.

As described earlier, in most plants, the first formed carbon compound in photosynthesis is the C₃ compound phosphoglyceric acid. However, in certain tropical plants like *Zea mays* and *Saccharum*, in addition to the C₃ pathway described, CO₂ is trapped through another pathway. In this process carbon dioxide is trapped by phospho enol pyruvate (PEP) to form a C₄ compound oxalo acetate. This compound is then converted to another C₄ compound, malate. In the bundle sheaths of the leaves, the malate is broken down to form PEP and CO₂ is released. This CO₂ now moves along the normal pathway of ribulose diphosphate (RuDP) with the end formation of a hexose sugar. In this way, in these C₄ plants, CO₂ is trapped along two pathways: 1. along the normal C₃ pathway with RuDP, and 2. along the PEP pathway. These so called C₄ plants fix a greater quantity of CO₂, resulting in the production of a greater amount of sugar, than do normal plants. For example, it has been found in the United States that the conversion of the incident solar energy into plant products in Soya bean, (*Glycine max*) which is a C₃ species, is only half that of a C₄ species like *Zea mays*. Though energy conversion in *Zea mays* is also below the theoretical maximum of 1.25 per cent, which might be sought in practical agriculture, even this increase, in doubling of production, would be of considerable significance in terms of food production.

The radiant energy trapped in the carbohydrates during photosynthesis by the green plants or primary producer is referred to as gross primary production (Gp). The gross primary production of a green plant increases according to its size. If we consider a succession on land, or a Xerosere, the first formed seral community has plants which have a small biomass. For example, if we take a forest land which has been cleared, cultivated and then abandoned, the very first seral plant community will consist of small herbaceous plants, mainly annuals. After a few months the dominant vegetation will change to slightly bigger herbs and later to shrubs, which are perennials. Ultimately, after many decades, the last or climax community will come up, comprising trees as the main life form. This type of succession may take a couple of hundred years. As a succession progresses it is seen that there is an increase in size of the plants comprising the seral community. This increase occurs until the climax, which has the largest plants is established. Therefore the gross primary production in a succession too increases till the climax is reached, at which point it remains more or less at a steady level. In green plants some of the energy fixed is stored as organic matter in new tissues. The amount of new tissues measured as dry weight is net primary production (NP). A part of the energy fixed is required to support the living plant. This is more than half the amount of energy fixed and is released during respiration to provide energy for the life processes of the plant. Parts of the plant like leaves, bark, fruit, etc. are lost to the environment. Therefore, in a plant, the total energy fixed, or gross primary production may be represented by the equation:

$$GP - R_{SA} = NP$$

GP is the gross primary production, R_{SA} is the energy used by the green plant (autotroph) during respiration. The amount left over is net production NP. The growth of the plant is measured in dry weight or energy stored which is net production.

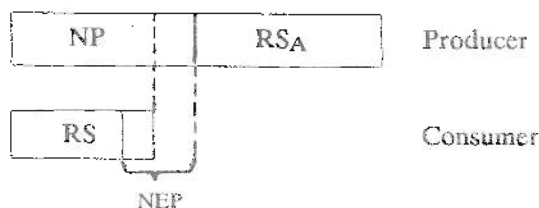
This can be further clarified by the following diagrams:

- (1) In a green plant, of the total energy fixed or gross production, a part is expended by the plant as Respiration (R_{SA}) and the rest goes into net production (NP).

NP	RSA
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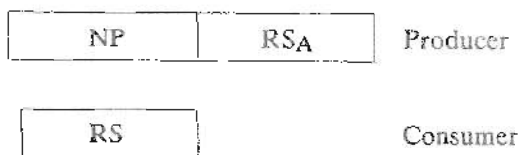
Gross Primary Production

- (2) In a successional plant community some of the net production is stored as growth and the rest (fallen leaf litter etc.) is used by consumers, which in turn expend most of it in respiration while storing some part as growth, adding to net ecosystem production (NEP).



Gross Production

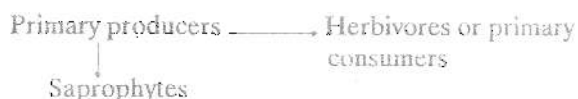
- (3) In a climax community the total energy fixed by primary producers is used in the respiration of the autotrophs and heterotrophs. Although plants, animals and reducers in the ecosystem go through the normal process of growth, the total energy fixed by the primary producers over a period of time is balanced by the respiration of the primary producers themselves together with the respiration of the animals and the saprophytes. Hence there is no net ecosystem production or NEP.



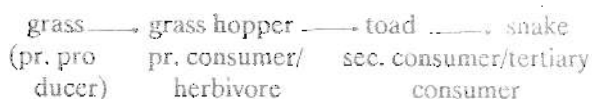
Thus we see a sharp distinction between a successional or developmental ecosystem and a climax or mature one. In a seral community the total respiration of the green plants is less than the gross production leaving energy (NEP) built into the structure of the ecosystem and added to the resources of the environment in which the plants occur. In a climax system all energy fixed is used in the combined respiration of the primary producers and heterotrophs as well as that of the organisms that live on the decaying organic matter.

There is no extra energy left and no net annual storage. Hence $NEP = 0$

The flow of energy through living organisms in a community is depicted by a food chain. Let us take the case of herbivores or primary consumers that feed on green plants (primary producers). These animals do not receive the entire energy fixed by the primary producers. As we have seen above, a good part of the fixed energy in primary producers goes to support the life processes of the producers themselves with only a small fraction going to build up the biomass. This small fraction is what is available for use at the next trophic level. The first level of consumers comprises herbivores like grasshoppers, caterpillars, deer etc. which feed directly on the green plants, and saprophytes which feed on the decaying leaves and other plant parts. This is illustrated in the following diagram.

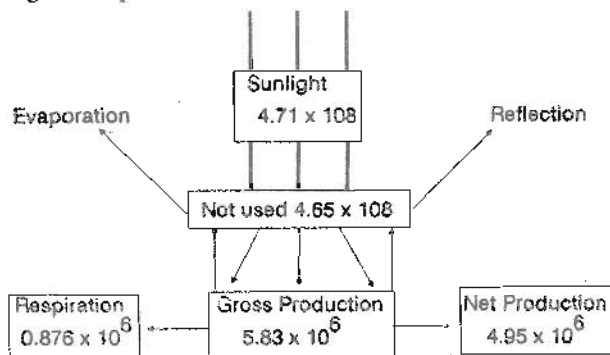


Thus the herbivores may obtain only the energy left after the energy expended for respiration of the green plants and the loss to decaying organisms. The energy available to the second trophic level of a food chain is the net primary production and is only about 10-20 per cent of the gross primary production. When herbivores become prey to carnivores at the next nutrient level of consumers, the energy received by the carnivores is only about 10-20 percent of that received by the herbivores. This is because about 80-90 per cent of the energy in the primary consumers is lost partly as respiration and partly as excretory material deposited in the environment. So, along a food chain there is depletion of energy. Let us take a typical predatory food chain where the energy flows from the primary consumer level, where the organisms are small, to larger organisms at the higher trophic levels. This is the most common type of food chain occurring in nature. An example is given below.



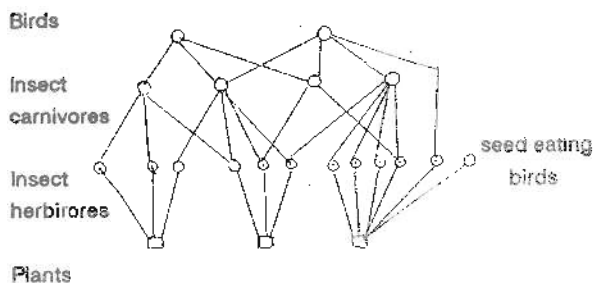
The grass (green plant) is the primary producer. Let us assume that gross primary production or energy

trapped during photosynthesis in the grass over a period of time is 1000 k cal. A large part of this energy is used up for the respiration of the plant and a small amount lost as decay of plant parts to the soil. The aggregate loss is nearly 80-90 per cent of the gross production. Therefore only about 10-20 per cent is available for the second nutrient level. Of the energy received by the grass hoppers (herbivores), that is, around 150 k cal, once the energy expended in respiration and the energy lost as excretory material to the environment by the grass hopper is excluded, the balance 10-20 per cent only i.e. around 15-30 k cal will be available at the third nutrient level of the toad or secondary consumer. In the toad, too, energy will be utilised in respiration and lost as excretory products to the environment so that only 10-20 per cent of what is received is available for the fourth trophic level of the tertiary consumer or snake. Thus, of the energy that was trapped by the primary producer i.e. 1000 k cal, there will only be approximately 3-4 k cal available for the tertiary consumer. From this we can deduce that if the energy flows along a single pathway then food chain could maintain only about 2 or 3 trophic levels of consumers, since there will be inadequate energy for higher trophic levels.



The fate of solar energy incident on perennial grass-herb vegetation in Michigan USA. All measurements in cal/m²/year

However, in nature, long food chains with a number of trophic levels have been recognised. This is possible because at higher trophic levels consumers, (snakes/toads) derive their inputs of energy from several food chains and not along a single food chain. For example, toads feed on a number of insects and not only on grass hoppers. Therefore predatory food chains are inter linked and joined by others to form what are known as food webs.



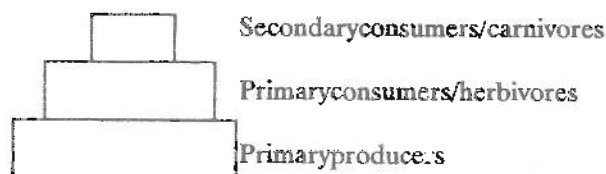
Hypothetical Food Web

Animals of different trophic levels are of different sizes. Any one large sized animal usually eats a variety of food, so that it is a part of several food chains. But food chains commencing from a plant source must always radiate outwards as the plant is used as food by different herbivores and these herbivores are in turn eaten by different carnivores. Thus a complex web is formed.

A food chain therefore can be defined as the path along which energy travels in nature. The energy which originally came from the sun does not go back to the sun. The energy flows along an open ended chain and is not cyclic. Thus energy flow in nature is an open system.

Other points to note in a food chain are the biomass and the numbers at each trophic level. In our previous example the largest animals were found at the nutrient level of the snakes, unless another predator ate the snake. The snake feeds on toads which live close to water and, though smaller in size, are present in greater numbers. The toads are insect eaters, their food being the grass hoppers and other small insects. These in turn feed on organisms smaller than themselves. As one works upwards along a food chain it seems that the animals become progressively larger but less abundant. A community can be separated into groups of animals of similar size. The animals in each of these groups would be feeding at about the same trophic level of a food chain. The smallest size class would have the largest number. If the number of individuals in each size class are represented in the form of a diagram with the first trophic level at the base one would get roughly a pyramidal figure. Each box represents a size class and would consist of animals feed-

ing at the same level of a food chain. This is termed the pyramid of numbers.



Although we have heard of food chains from primary producer to primary and secondary consumer and so on, there is another important part of the food web which has not been considered. All organisms, whether primary producer or consumer, eventually die and the dead organic matter is decomposed by saprophytic organisms like bacteria and fungi. In addition, nitrogenous compounds are excreted to the environment and are broken down by saprophytic organisms. In some cases, where animals die, a greater part of their bodies are eaten by scavenger animals. Ultimately the scavenger chain ends also with the saprophytic organisms which break down all the complex compounds into simple molecules and releases them into the environment. In this type of food chain energy flows along the dead substrates to the living scavengers and can be termed as a decomposer or saprophytic food chain.

In other instances organisms live parasitically on the host, and energy is diverted along a parasitic chain. When a parasite is in turn parasitised by another organism e.g. a protozoan living on a tick which is living parasitically on a cow, the energy passes into the protozoan. If there are a number of *Cuscuta* plants on a single tree, and they are in turn parasitised by worms, this could be a parasitic food chain. In these food

chains, as in the scavenger food chains, the organisms at a particular higher trophic level are smaller than their host, or the previous level, and the niches they occupy require that they be smaller than the organisms from which they derive their food.

In nature, the direct rays of the sun heat up the earth. As stated earlier a good part of this energy goes back into space as infra red energy. But due to man's activities in industry, and in the clearing of forests, this delicate balance is being upset and there is a build up in the concentration of gases like carbon dioxide, oxides of nitrogen and sulphur in the atmosphere. These gases trap the heat in the atmosphere preventing a part of the energy being re-radiated into space, thus causing global warming. This is called the "green house effect". Carbon dioxide levels are 25 per cent higher now than a century ago and the amount of carbon dioxide in the atmosphere seems to be increasing. By the middle of the next century global mean temperatures could rise from between 1.5° to 4.5° Celsius. The result of this would mean the melting of glaciers and the ice at the poles, and the warming of the sea causing expansion of the water, and these in turn will cause a rise in the sea level which may be as much as two or three metres. The result would be an inundation of low lying coastal areas and islands all over the globe. Unless remedial measures are taken eg. forest restoration programs and cutting down in the use of fossil fuels, the effect will be harmful to mankind.

In conclusion, we could recognise that the radiant energy of the sun is trapped by green plants. This energy is passed along food chains in nature. A greater part is reflected back into space as heat and light. This energy flow in nature is not a closed system.