IICA

GUIDELINES FOR THE IDENTIFICATION AND DIAGNOSIS OF DAMAGE IN CROP PLANTS CAUSED BY INSECTS, DISEASES, WEEDS AND NUTRIENT DISORDERS

Edited by: Chelston W.D. Brathwaite
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Ministry of Food Production,
Marine Exploitation, Forestry and the
Environment of Trinidad and Tobago

University of the
West Indies

Port-of-Spain, Trinidad & Tobago
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A document based on presentations made at the first regional training course on the diagnosis of plant pests and diseases of food crops held at the Faculty of Agriculture, The University of the West Indies, St. Augustine from July 7th to 12th, 1986 and sponsored by the Faculty of Agriculture, U.W.I., the Ministry of Food Production, Marine Exploitation, Forestry and the Environment of Trinidad and Tobago and the Inter - American Institute for Cooperation on Agriculture (IICA)

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PREFACE

The aim of this document is to satisfy the need for a source of information which could form the basis for training courses on the identification of plant pests and diseases of crops of economic importance in the Caribbean. It is believed that the inability to accurately identify pest species and to diagnose disease problems in the field is one of the most important limiting factors in effective pest control in the Caribbean. This document resulted from presentations at a regional seminar on the subject which was designed to increase the level of skills of persons responsible for the diagnosis and control of plant pests and diseases in various countries of the region.

The course emphasized the practical techniques which are required for correct pest and disease diagnosis and utilized local examples in the laboratory and in the field. The course lasted for one week and was led by specialists in pest and disease diagnosis and control from the University of the West Indies, Faculty of Agriculture, the Caribbean Agricultural Research and Development Institute (CARDI), the Ministry of Food Production, Marine Exploitation, Forestry and the Environment, and the Inter-American Institute for Cooperation on Agriculture (IICA). The course was conducted in English.

The course was attended by thirty-two participants including representatives from Barbados, Grenada, Haiti, St. Lucia, Dominica and Jamaica. The course was declared open by the Permanent Secretary, Dr. E. Patrick Alleyne and at the closing ceremony Dr. Federico Dao, Director of IICA Plant Protection Program and Dr. Alleyne presented certificates to the participants a list of whom is presented in the appendix to the document.

The material is presented on the basis of causal agents e.g. fungi, bacteria etc. as this was envisaged as the most practical way of presenting the data but the editors are aware that problems in the field are multi-disciplinary and this was emphasized throughout the course and especially during the field trips. The editors are aware that this document would be far more valuable if photographs were used to illustrate the text but cost constraints limited this approach.

The course presentations were evaluated by the participants and their views were taken into consideration in the preparation of the final version of the document.

It is hoped that a companion laboratory text with photographs will be the next stage of this project. Steps in this direction have already been taken.

The editors hope that this introductory text will contribute to more accurate diagnosis of plant pest and disease problems in the Caribbean and thus assist in improving pest control in the region.

ACKNOWLEDGEMENTS

The editors acknowledge the contributions and comments of the participants in the First Regional Course on the Diagnosis of Pests and Diseases of Food Crops in the Caribbean who came from the Ministry of Food Production, Marine Exploitation, Forestry and the Environment; Faculty of Agriculture, UWI; Food and Agriculture Corporation; Caroni (1975) Ltd. and the Ministries of Agriculture of Barbados, Dominica, Haiti, Grenada, Jamaica and St. Lucia.

The editors also acknowledge the approval of the following institutions to use the figures in the manual designated after their names: Academic Press Inc. - Figure 1 page 31 and Figure 2 page 33 from Plant Pathology by G.N. Agrios; W.H. Freeman and Company for Figure 3 page 36 from Fundamentals of Plant Pathology by D.A. Roberts and C.W. Boothroyd; University Press Ltd., Nigeria for Figure 1 page 12 from A Laboratory Manual of Entomology by A. Youdeowei; Holt, Rhinehart and Winston for Figures 2A, 2B, Figures 3A, 3B page 13 and Figures 4A, 4B page 15 from An Introduction to the Study of Insects by D.J. Borror, D.M. DeLong and C.A. Triplehorn; Academic Press Inc. (London) Ltd. for Figure 4C page 15 from Thrips by T. Lewis.

Technical and editorial support of Sharida Hosein and the secretarial assistance of Francilla Stewart are also acknowledged.

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Chelston W.D. Brathwaite

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THE PRINCIPLES AND IMPORTANCE OF THE DIAGNOSIS OF PLANT PESTS AND DISEASES

by

Chelston W.D. Brathwaite

1. INTRODUCTION

Plants, like animals are exposed to a number of injurious circumstances which reduce their growth and development. In our efforts to produce healthy plants which produce maximum yields, it is important to be able to determine when such injurious circumstances are occurring and to develop the means and methods to effectively remove or reduce the effect of unfavourable conditions. Creation of the ideal environment for plant growth implies a thorough knowledge of the requirements for ideal growth and the production of maximum yield of the crop.

When the crop is not producing at its maximum then we must investigate why and apply corrective or remedial measures.

The process of determination of the cause or causes of unfavourable circumstances on the growth and production of crops can be referred to in the broad sense as "diagnosis". Diagnosis as used in this context implies an investigatiive approach to the problem.

In any consideration of diagnosis, one is first concerned about the nature of the deleterious effect. It is biological? is it caused by some physical agent? or is it caused by the lack of some element or principle which is required by the plant for healthy growth and normal development.

In view of this broad concept of diagnosis, the investigator must be aware of the ecological, biological and physical requirements of the plant and be fully cognizant of what may be considered as "normal".

In order to determine what is abnormal, familiarity with the normal is very important. The investigator must be aware of cultivar characteristics, methods of culture and environmental requirements for normal growth etc. To use an example from the medical profession - if a baby is taken to a General Practitioner one of the first things the medic may do is to weigh the child. If the weight of the child is less than what it should be for a child of his/her age (normal weight) the medic proceeds to investigate or diagnose the reason for the abnormality and having come to some conclusion based on a variety of criteria, he proceeds to prescribe a remedy. The process of correct diagnosis is therefore absolutely necessary for the prescription of a correct control measure or a remedy.

The process of learning what is normal and what is abnormal for food crops requires considerable experience. This course is designed to reduce this time span by tapping the experience of eminently qualified professionals who have worked with these crops.

2. SOME IMPORTANT ELEMENTS OF DIAGNOSIS

a. an incorrect diagnosis and therefore an incorrect recommendation is usually more costly than doing nothing

b. it is better to say what it is not and try to investigate what it is than to guess the cause

c. the environmental conditions prevailing during the growth of a crop may be very important in attempting diagnosis

d. Diagnosis often requires observation of rather subtle differences between normal and abnormal plant growth and a keen observation is vital

e. Diagnosis requires an open mind, a broad perspective on the crop and a knowledge of plant science, soil science, meteorology and plant pathology, entomology, nematology and weed science

f. Diagnosis requires that the concept of multiple causation and interaction be borne in mind at all times especially in the consideration of problems affecting plant roots. For example, stunting, wilting, uneven growth, yellowing are all non specific symptoms which may be caused by biotic or abiotic agents or a combination of these. They may be due to insect feeding, herbicide damage, nutrient deficiency or excess or a variety of other biotic agents.

The process of diagnosis of problems of crop growth may be divided into three stages.

1. Field Examination

2. Laboratory Examination

3. Field Plot testing

3. FIELD EXAMINATION

Field examination is the common experience among extension personnel who are called by the farmer to examine his field where he has a problem with one or more of his crops. The farmer of course wants to know primarily what is the solution to the problem but solutions to problems can only be adequately addressed when the cause of the problem has been correctly diagnosed.

What approach should be taken?

While it is generally difficult to prescribe a specific set of actions which one should take in a field situation a few general guidelines can be given.

1. Determine, if possible, the site of primary injury e.g. above ground or below ground

2. Determine the distribution of the symptoms e.g. is it random or along the rows

3. Determine the symptom distribution in relation to wind direction e.g. is it on the windward or the leeward side

4. Determine symptom distribution in relation to the topography of land e.g. is symptom in low lying areas or on high ground

5. Consult a recognised text on the crop and compare descriptions of deleterious circumstances with those in the field

In any attempt to assess the cause of conditions in the field, one must bear in mind the complexity of the soil environment. Soil has been described by one ecologist as the most complex ecological system on earth. There are a wide range of possible biotic and abiotic interactions occurring in soil which may be neutral, beneficial or detrimental to root growth and function and consequently to plant growth and yield.

Visual diagnosis, however, may only lead to a partial or preliminary answer. In order to obtain the true answer laboratory diagnosis is important whether this is tissue analysis for nutrient deficiencies or isolation for biotic pathogens.

4. LABORATORY EXAMINATION

With the clues obtained from the field situation, laboratory examination of samples and attempts to isolate the pathogens or to observe the insect or to identify the nutrient deficiency or excess is necessary.

5. SAMPLE COLLECTION

The collection of a representative sample which reaches the laboratory in the desired state is very important. Samples should be collected from plants showing different stages of the symptom but the sample size and type will depend on the use for which the sample will be used. The kinds of samples and methods of collection and laboratory procedures will be considered by the various specialists under their respective sections.

Details of some laboratory procedures as these relate to the diagnosis of plant disease are dealt with in some detail in my introductory text (An Introduction to the Diagnosis of Plant Disease IICA, 1981).

6. KOCH'S POSTULATES

Koch Postulates or Koch Rules of Proof of Pathogenicity are a set of rules which were developed by pioneer bacteriologist Robert Koch to assist in the determination of the causal agent of a disease. The postulates state that in order for an agent to be considered the cause of a disease situation the following must be satisfied.

1. Associate the organism with all cases of the disease

2. Isolate the organism and grow it in pure culture

3. Inoculate susceptible plants with the isolated organism and obtain identical symptoms to those previously observed

4. Re isolate the organism compared with the original and they should be identical

While Koch's Postulates have stood the test of time and have been a very useful tool in plant pathology, within the context of the broad view of diagnosis which I have outlined, the postulates would not be applicable in some cases. For example:

a. in consideration of nutrient deficiencies and nutrient excesses the idea of isolation of the causal factor and growing in culture is impossible

b. in situation where the agent cannot be grown in pure culture then the postulates cannot be applied

c. the situation of multiple causation which is common in the field is not compatible with the postulates

d. the postulates cannot be applied to environmental influences such as toxic chemical and gases

e. the damage caused by insects cannot be analysed in this way

f. even in the application of the postulates in the traditional way for plant pathogens care must be taken when negative results are obtained. It is very difficult to reproduce field conditions in the greenhouse or laboratory and lack of symptoms on inoculating a plant with a pathogen may only be a reflection of our inability to reproduce the strict and demanding environmental conditions required for pathogenicity and symptom expression.

7. FIELD PLOT TESTING

It is not unusual to use field testing as a method of verification of the cause of abnormal plant growth. In these circumstances, field plots

are laid out and various treatments are applied. In so doing, it is important to ensure that satistical methods are employed and that the necessary dupication and randomization of treatments occur. With respect to work with fungicides, nematicides etc. which may be used to control a suspected primary agent. The following should be observed:

a. chemicals may affect growth of the plant and yield quite apart from controlling a specific pest or disease

b. chemicals may control organisms other than the target species

c. representational error due to interplot interference may arise

These effects may have the potential to complicate the results of the experiment

What is interplot interference?

Interplot interference can arise in field experiments involving plant pests and diseases only when the following two conditions exist (a) the plots have different levels of infestation or infection (b) the pathogen or pest is capable of moving from one plot to the next. In this situation the plots cease to be independent of each other. This lack of independence violates the cardinal rules of experimental design and analysis and consequently the interpretation of the results is difficult. James (1979) has recommended that this must be considered in all loss evaluation with wind borne pathogens and has set out methods for overcoming interplot interference.

8. RECOGNITION, DIAGNOSIS AND PEST CONTROL

Losses due to plant pests, diseases and weeds continue to reduce yields and increase the cost of food crop production. A generalized estimate of these losses is usually placed at 30% but this is a generalization and in some cases the loss can be 100%. In addition, the cost of control has escalated in recent times owing to the increased cost of pesticides, increased labour costs, and increased variety of pests and diseases.

Maximum yields of crop plants can only be obtained by providing solutions to the problems which may limit growth and development. Correct diagnosis of the problems can lead to corrective measures thus making it possible to obtain maximum yields.

9. THE COST OF INCORRECT DIAGNOSIS

a. Pesticide misuse: There is general concern about the use of pesticides as they affect man, animals and the environment. Incorrect diagnosis will lead to pesticide misuse as insecticides may be used to solve nematode problems and fungicides may be used to control bacteria. Fertilisers may be added to virus-infected plants if the persons involved is of the view that the symptoms observed are due to a nutrient deficiency.

b. Ineffective eradication of new pests and diseases: Lack of diagnosis or recognition can lead to the establishment and spread of new pests. If new pests and diseases are recognized early eradication programmes can be established resulting in effective control e.g. Moko disease.

c. Destruction of Confidence: Wrong diagnosis will invariably result in a continuation of the problem with the consequent loss of confidence in the extension officer.

d. The cost of wrong diagnosis:

1. loss of crop

2. more costly control

3. national and international spread of diseases

e. Conclusions:

1. In diagnosis there is no replacement for experience therefore, get all the exposure you can to the crops and their methods of growth.

2. A diagnostician must be a generalist or of such breadth of knowledge that he/she has a general overview. The specialist however, has an important role to play as the world of information is far too broad and deep for any one person to have all the wisdom in all the disciplines which are needed to diagnose plant pests and diseases. Symptoms are generally non-specific e.g. the yellowing of the leaves of a plant may be due to:

1. Herbicide damage
2. Phytotoxic effects
3. Toxic gases
4. Infection by fungi
5. Infection by viruses
6. Infection by bacteria
7. Nematode attack
8. Physical damage
9. Nutrient deficiencies
10. Insect damage

And finally a word of advice from Streets "Don't bluff" be prepared to say "I don't know and I will find out for you"

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PLANT PEST DIAGNOSIS - SYMPTOMS OF INSECT AND MITE ATTACK ON CROP PLANTS

Gene V. Pollard

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PLANT PEST DIAGNOSIS - SYMPTOMS OF INSECT AND MITE ATTACK ON CROP PLANTS

by

Gene V. Pollard

1. INTRODUCTION

The essential function of plant protection is essentially to manage or control pest populations to sub-economic levels by the utilization of various strategies which cause either increased mortality or decreased natality in the pest population. The former is usually achieved through the use of chemicals or by cultural and biological control techniques. More recent techniques like the sterile insect techniques serve to decrease the birth rate of the pest population. The end result is the same - a reduction in the pest population. Other strategies attempt to prevent the pest from attacking the crop through the use of protectant chemicals or by legislative means, mainly plant quarantine procedures. The overall aim of all these various strategies, of course, is to increase crop yield and quality.

Whatever the practice, however, efficient pest control ultimately depends on an accurate diagnosis of the pest problem. This is especially important where one may only be observing symptoms of pest or disease damage and not the organism responsible for such damage. While plant pathologists may and, in fact, do frequently make an initial diagnosis of a disease problem solely on the appearance of damage symptoms on the plant or organ prior to subsequent verification by laboratory investigation, similar diagnosis of insect pest damage has not been as commonly practised by entomologists. In fact part of the facilities of any good crop protection unit will be a "Plant Disease Diagnostic Laboratory". There is never an equivalent in the entomology section. Diagnosis is also part of the curriculum of any plant pathology course.

Despite all that has been said, however, any experienced field entomologist, will readily admit, I am sure, that a study of damage symptoms on the host plant represents, even unconsciously, the first step in assessing any insect pest problem. Such diagnosis may be sufficiently distinct in some instances to allow for the identification, with a great deal of certainty, of even the genus responsible for the observed damage and usually at least of the likely pest group. For example, damage resulting from leaf-cutting ant attack cannot be confused with damage from other leaf-feeding insects. Similarly, leaf miner damage is very conspicuous.

Perhaps a more important consideration for the entomologist is to determine whether an insect observed on a crop plant is a pest or not. Although there are probably close to one million identified insect species the majority are not pests. On the contrary, many are beneficial to the farmer, serving either as natural enemies of various pest species or as important pollinators. One therefore must study the biology of a species and its relationship to the plant to determine the economic status of an insect. In this respect the type of mouthparts of any insect species and, consequently, the manner of feeding on the host plant are both important determinants of its pest status. Hence, diagnosis of insect damage is facilitated by a knowledge of insect mouthparts and the feeding mechanisms observed.

2. INSECT MOUTH PARTS AND HOST PLANT FEEDING

Insects may attack any part of the plant and the nature of damage resulting is related primarily to the type of mouthparts of the pest species. Often characteristic damage patterns are observed.

Mouthparts of pest species may be classified as biting and chewing, rasping or piercing and sucking. A species may have two different types of mouthparts in the mature and immature stages. Frequently it causes damage to a host plant in only one of its life stages.

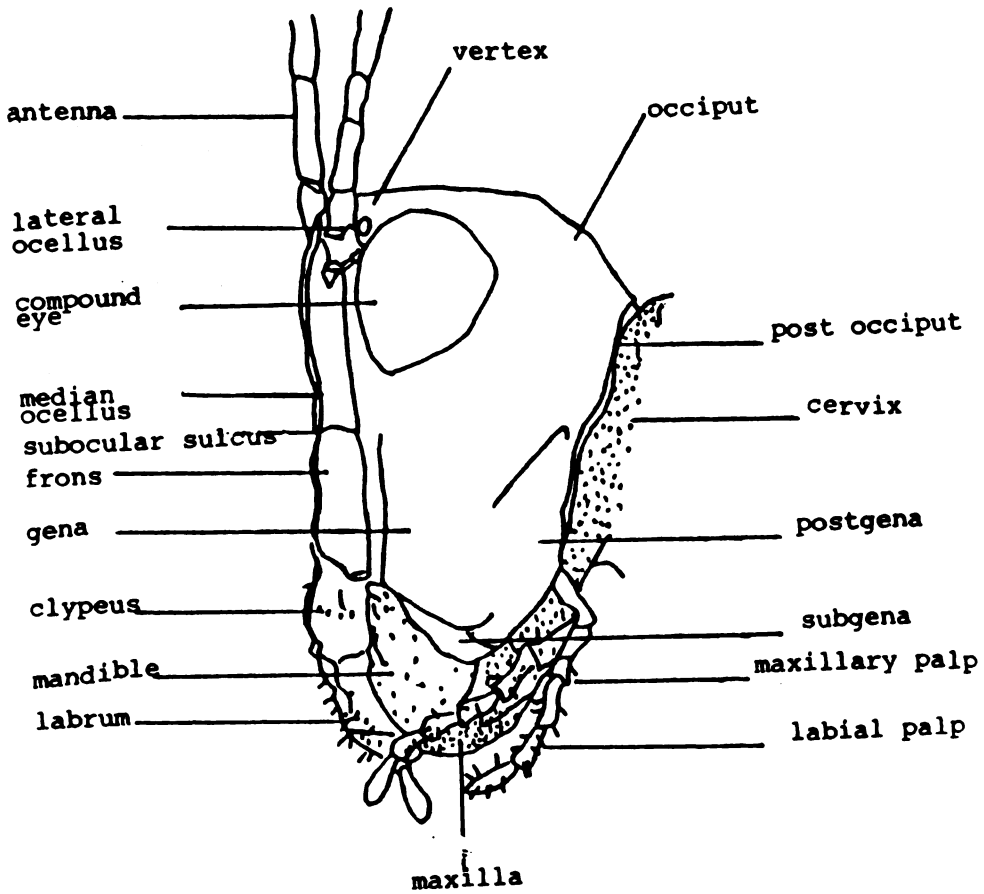
2.1 Biting and Chewing Mouthparts

This type of mouthpart is generally regarded as the most primitive or basic type seen in insects (Figure 1). All other types are thought to have evolved from these. Biting and chewing mouthparts may be found in both the mature and immature life stages in beetles, grasshoppers, crickets, locusts. In some other instances these mouthparts are confined to the immature stage. Adult lepidoptera, for example, are not generally damaging to host plants since they feed on nectar sucked up through a tube-like proboscis (Figure 2); the immature stage or caterpillar, on the other hand, has biting and chewing mouthparts.

Insects with these mouthparts represent some of the most destructive of pests. In this group are a number of leaf-feeding species capable of completely defoliating plants as well as various stem, root, shoot and seed boring species.

2.2 Piercing and Sucking Mouthparts

Insects with these mouthparts belong to the order Hemiptera and represent one of the most, if not the most, important economic pest groups. Here all or most of the individual mouthparts as seen in biting and chewing insects have become modified to form needle-like stylets (Figure 3). These form a hypodermic-like structure capable of both piercing the plant surface and ingesting or sucking sap from inside the plant. Saliva is usually injected into the plant when the surface is pierced. The plant suffers severe damage as a result. Direct



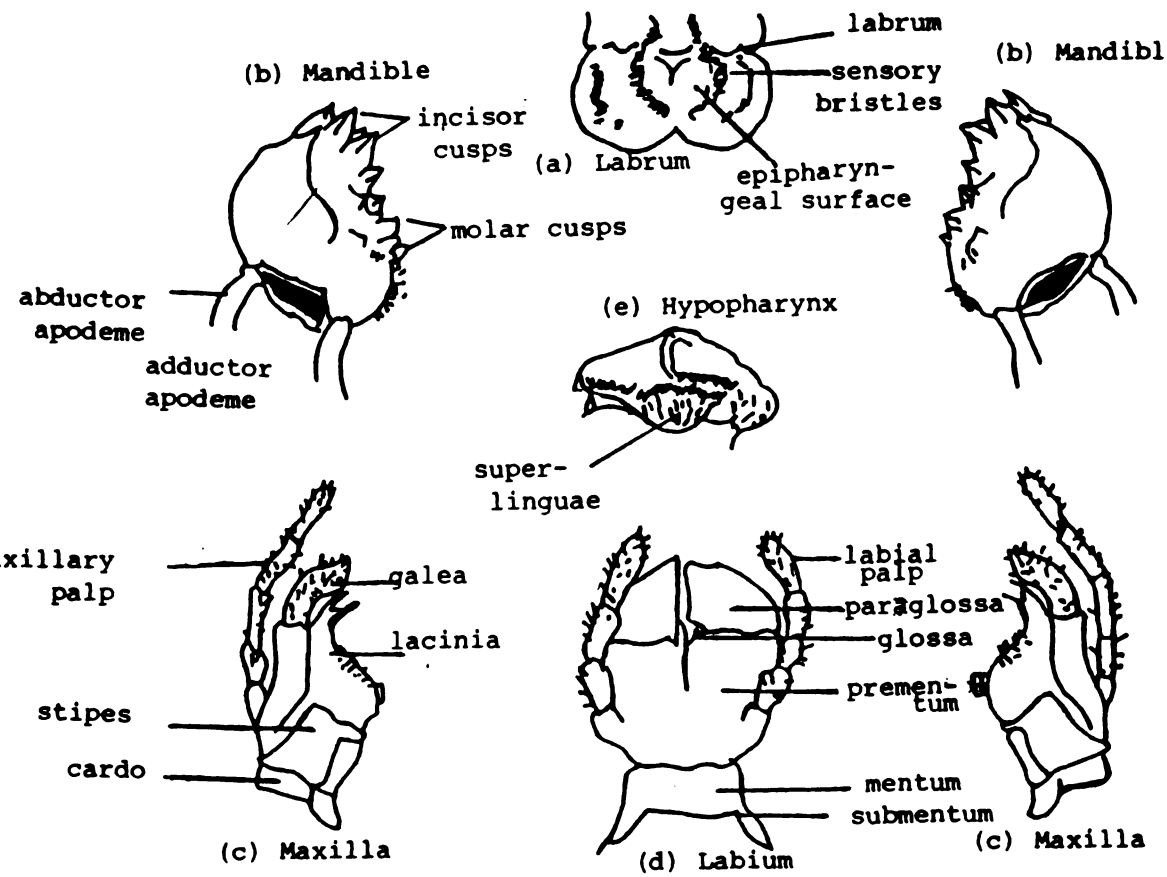


Figure 1 Head and Mouthparts of a locust (from Youdeowei, A. - "A Laboratory Manual of Entomology", 1977).

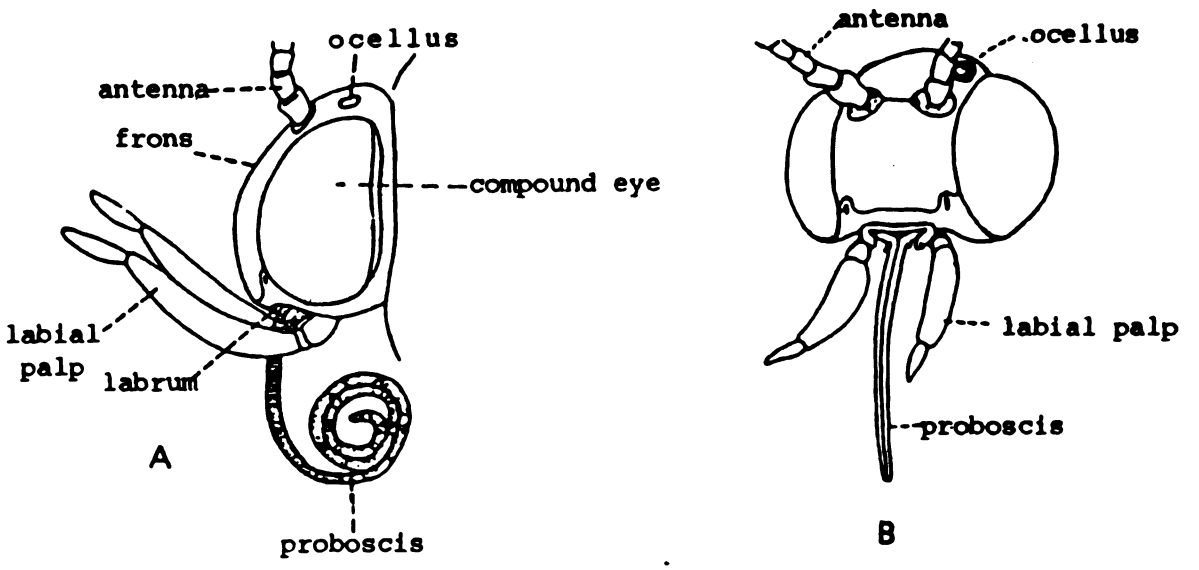


Figure 2 Mouthparts of a lepidopteran insect. A - lateral view of head; B - anterior view of head.

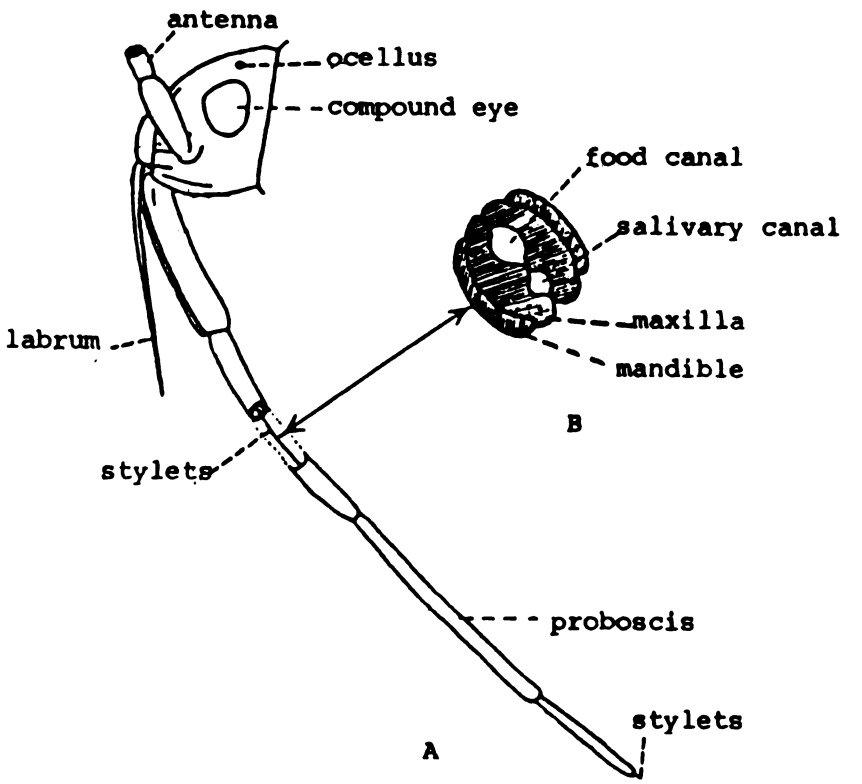


Figure 3 Mouthparts of the large milkweed bug Oncopeltus fasciatus (Dallas). A - lateral view of head; B:- c.s. of stylets.

(All diagrams from BORROR, D.J., D.M. DE LONG and C.A. TRIPLEHORN - "An Introduction to the Study of Insects" 4th Edn, 1976.

damage results both from the loss of plant sap as well as to toxicogenic effects of the injected saliva while indirect damage may result from the transmission of plant pathogenic organisms during feeding.

Both adult and immature hemiptera have piercing and sucking mouthparts, attacking the same host plant. Among these pests are included stink bugs, aphids, leaf and plant hoppers, scale insects and mealy bugs.

2.3 Rasping Mouthparts

These mouthparts are found in thrips (order Thysanoptera). These are quite small insects although they range from 0.5mm to 14mm. Their mouthparts are arranged in what is referred to as a mouth cone beneath the head but usually seen near to or between the base of the fore limbs (Figure 4).

The most usual feeding action is for the insect to firmly place its mouth-cone against the plant surface, insert the mandible into the plant and then rock its head upwards and downwards; the mandible rasps or tears the epidermal cells. The maxillae then break the walls of the mesophyll cells and the expressed sap is sucked. Both adult and immature insects feed in this fashion.

3. DIAGNOSIS OF INSECT DAMAGE IN CROP PLANTS

Apart from some general symptoms described above resulting from insect feeding, there are more specific symptoms which may be used to assist in the diagnosis of pest damage and even to the possible identification of the culprit species.

Table 1 is a descriptive listing of major phytophagous species based on the site of damage, as well as the type of damage, to the host plant. Using these two criteria it is possible to diagnose host plant damage based on the following:

(i) whole-plant symptomatology

(ii) leaf damage

(iii) stem/shoot and root damage

(iv) flower and fruit damage

These, of course, will not be totally unrelated since severe stem or root damage may be reflected in reactions of the plant as a whole. However, the above classification is a convenient way to attempt to diagnose host plant damage.

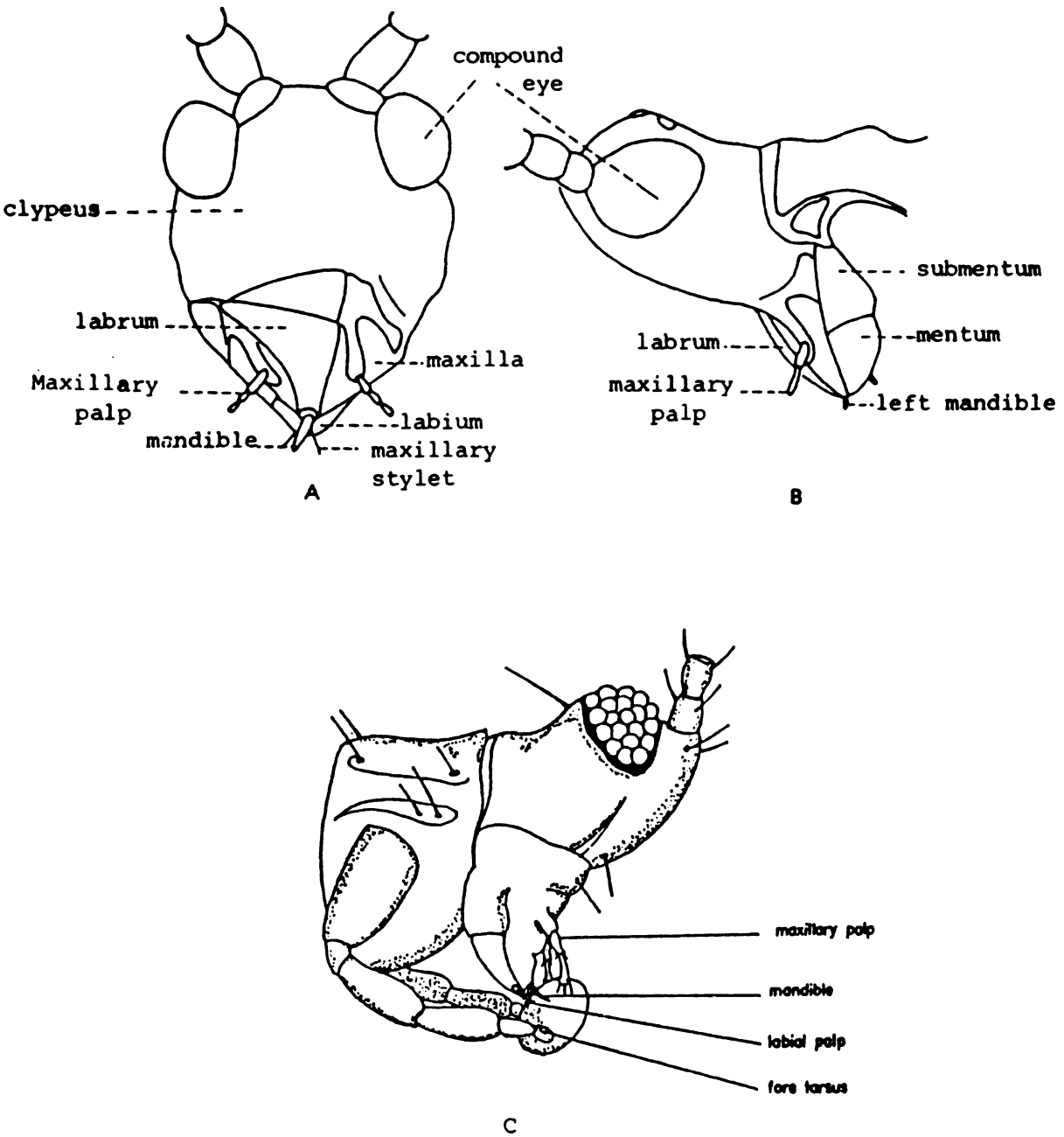


Figure 4 Mouthparts of a Thrips

A Head, ventro-anterior view

B Head, lateral view

C Thrips feeding on a pollen grain

(A,B from Borror, D.J., D.M. DeLong and C.A. Triplehorn - "An Introduction to the Study of Insects", 4th ed. 1976, C - from Lewis, T. "Thrips" 1973)

TABLE 1. Major phytophagous insect pest species (modified from Daly, H.V., Doyer, J.T. and Ehrlich, P.R. (1978), Introduction to Insect Biology and Diversity)

1. EXTERNAL FEEDERS

(i) Exposed Feeders

Isoptera (some termites)

Orthoptera (grasshoppers, crickets, locusts)

Hemiptera - Heteroptera (e.g. stinkbugs; lacewings)

Hemiptera - Homoptera (e.g. aphids; leafhoppers; planthoppers; scale insects)

Thysanoptera - (thrips e.g. banana thrips)

Lepidoptera (many leaf-feeding caterpillars e.g. army worms; diamond-back moth)

Coleoptera (e.g. leaf beetles; flea beetles; cutworms)

Hymenoptera (e.g. leaf-cutting ants)

(ii) Leaf Rollers

Lepidoptera (e.g. arrowroot leaf roller)

2. INTERNAL FEEDERS

(i) On Foliage, Stem and/or Roots

Borers - various lepidopteran, coleopteran and dipteran species (e.g. sugarcane stem borers; sweet potato borer; banana borer; coconut weevil)

Leaf miners - various lepidopteran, coleopteran and dipteran species

Gall formers - dipteran species mainly; also lepidopteran and coleopteran species (e.g. gall midges)

(ii) On Fruits and/or Seeds on Living Plants

Diptera - tephritid fruit flies (e.g. West Indian Fruit Fly; Guava Fruit Fly)

Lepidoptera - various pod borers on beans/peas; fruit piercing moth

Coleoptera - seed weevils; fruit scarring beetles

Hymenoptera - fruit piercing bee

3.1 Whole-plant Symptomatology

At times it may almost be impossible to relate a particular plant malady with insect pest attack. Symptoms of fiddler beetle *Exopthalmus spp.* attack on citrus in Jamaica, for example, include reduction in leaf and fruit size with the tree sometimes bearing a large number of small fruit out of season. One may be inclined to associated such conditions primarily with some kind of nutritional or soil deficiency or other agronomic factors and not with severe root attack by fiddler beetle larvae!

Generally though there are some symptoms of insect pest damage which are expressed through an affliction of the whole plant. These are:

3.1.1 Wilting/die-back. This may be the result of both direct and/or indirect insect attack of the host plant. Direct damage to root systems by mole crickets or subterranean cutworms is frequently the cause of death to the whole plant or part of the plant.

Indirect damage occurs when the pest is a disease vector, the pathogen being responsible for the actual wilt. The mechanism of hemipteran feeding is an ideal one for disease transmission. Saliva which is injected into the plant during feeding can carry pathogenic organisms. In fact the major vectors of plant diseases, particularly viral and bacterial diseases, are hemipteran insects. Major diseases such as Lethal Yellowing of coconuts, Bunchy Top of papaya, Cowpea Mosaic Virus diseases are all vectored by hemiptera.

3.1.2 Toppling. This results from a weakening of the basal parts of the plant. The main pest groups responsible for this are borers, cutworms and mole crickets. The Banana Borer, *Cosmopolites sordidus* Germ., tunnels through the corm of the banana plant and so destroys the tissue that the plant becomes very susceptible to toppling particularly when fruiting or during windy conditions.

Cutworms and molecrickets feed on the host plant at ground or subterranean level. Seedlings particularly may be so attacked that they fall to the ground.

3.1.3 Blighting or Burning. This type of damage is visible as a browning of the leaves of the host plant resulting from attack by sap-feeding insects. With heavy infestation levels the complete plant appears brown. A good example of this type of damage is seen in sugar cane when attacked by the Sugar Cane Froghopper, *Aeneolamia varia saccharina* Dist. Adult froghoppers feed on the leaves of the host plant. The damaged plant is said to be burnt with the condition being referred to as "froghopper blight".

3.1.4 Secondary infestations. Resulting from some primary or initial pest attack some plants become susceptible to secondary pest infestations. Bacterial and fungal infections frequently follow attack by boring insects leading to stem rots or root rots. One very widespread example of secondary infestation is the growth of sooty mold fungus on many plants.

Plant sap on which hemipteran insects feed is mainly a sugar solution with some amino acids and proteins. In order to obtain adequate amounts of protein much more plant sap than required must be ingested. Hence they must excrete the excess sap or honeydew as it is now called. It is excreted onto the plant surface on which the pest was feeding and is an ideal medium for fungal growth.

Plants which are attacked by piercing and sucking insects will therefore usually have their leaves covered with black growth of sooty mold fungus. This of course will reduce the photosynthetic potential of the plant.

Hence though one may not always readily observe aphids or scale insects on a plant the presence of the very visible sooty mold is a very good diagnostic indicator that such pests are active.

The presence of honeydew also serves to attract several ant species which may aggravate the pest situation since these ants may protect the pest from attack by natural enemies.

3.2 Leaf Damage

This may take different forms:

3.2.1 Chewed leaves. A variety of different insect species belonging mainly to the Lepidoptera, Orthoptera, Coleoptera and Hymenoptera can damage leaves of host plants with their biting and chewing mouthparts. It is not always easy to diagnose specific causal organisms responsible for chewed or bitten leaves. Most lepidoptera, for example, simply remove portions of leaf lamina in no apparent fashion. However, depending on the crop plant one may speculate, with some degree of assurance, on the likely pest organism.

Army work *Spodoptera* sp. damage on corn, for example, is seen as large irregularly shaped holes in the leaf running mainly parallel to the leaf edges. On the other hand, beetle damage may be observed as a number of small fairly neat holes scattered over the leaf blade. This is referred to as "shot hole" damage and is caused by the flea beetles or chrysomelid beetles. This type of damage is seen in crucifers and legumes (beans). Perhaps the most characteristic type of leaf damage

results from leaf-cutting ant attack (also called 'bachacs' or "acoushi ants"). These insects always cut from the outer edge of the leaf removing very neat semi-circular pieces.

3.2.2 Leaf mining. Insects causing this damage are the larval forms of Lepidoptera (Lyonetiidae, Gracillaridae) Coleoptera (Chrysomelidae) Diptera (Agromyzidae).

These larvae feed on the mesophyll leaf tissue leaving the vascular tissue and epidermis intact. Clear areas are observed on the leaf and these may take on one or two characteristic forms. With some species the larvae eat their way through the tissue in tunnel-like form changing direction when a leaf vein is encountered. A twisting or serpentine trail results. In other instances the larvae eat both the mesophyll and vascular tissue. An irregular area is removed which is referred to as a blotch.

3.2.3 Leaf malformations. In response to insect and mite damage (feeding or ovipositioning) some host plants develop abnormal tissue growths. Gall formation is one of the most extreme forms of malformation and may be found on various parts of the plant; but usually a particular insect will produce a characteristic type or gall (in colour and design, for example) on a particular part of the plant. Inside the gall may be present one (usually) or more than one insect.

Cassava plants usually have well developed leaf galls caused by midges (cecidomyiid flies). These galls are usually red to yellow in colour and appear as slightly curved cylindrical outgrowths projecting as much as 1cm or so above the upper leaf surface. Inside, the gall is hollow and contains a single yellow larva of *Jatrophobia brasiliensis* (Ruebsaamen), one of the most common species. On the lower leaf surface a single exit hole is observed on a slightly elevated ring of tissue.

Another form of abnormal growth is the very characteristic leaf curling seen in many plants as a result of aphid feeding. Usually young leaves or the new flushes are attacked and as these grow they assume a twisted or curled, misshapen form.

3.2.4 Scarring/Discolouration. Some types of leaf damage appear as a scarring or discolouration due to the effects of insects with piercing and sucking or rasping mouthparts (Hemiptera and Thysanoptera respectively). One effect of hemipteran insect feeding is a necrosis of the tissue surrounding the feeding puncture, visible as a yellow-brown spot on the leaf surface. With high pest numbers these spots may all coalesce so that the whole leaf becomes discoloured, dies and eventually is shed. This means a reduction or loss of photosynthetic potential of the plant. Retardation of growth results.

Symptomatic discolouration of the leaf of the host plant also results from thrips feeding. Leaves develop a "... silvery sheen due largely to air occupying the emptied cell cavities" (Lewis, 1973) after the cell sap has been ingested (see above). Heavy infestation of this pest leads to leaf fall. In some instances faecal droplets are also seen deposited around feeding sites. Fungal growth occurs on these droplets leading to discolouration of host leaves.

3.3 Stem/Shoot and Root Damage

Damage to stem/shoots and roots results primarily from the activity of boring insects, mainly larvae of lepidoptera and coleoptera although dipteran and hymenopteran larvale may also bore into plant tissue. As mentioned above, insect borers have biting and chewing mouthparts with which they eat and tunnel their way through stems and roots destroying at times large portions of the internal tissue. In many instances there is no external evidence of host damage except in some cases where exit or entry holes into the host are observed.

Shoot borers destroy the growing tip of the host. This may produce a characteristic damage pattern in some host plants. The sugar cane borer, *Diatraea* spp., for example bores into and kills the growing tip and inner leaf whorl of young sugar cane plants. As a result the whole dead shoot may then be very easily pulled out or removed from the stalk. This is called the "dead heart" condition and is quite diagnostic of *Diatraea* infestation in young sugar cane plants.

More generally, borer damage is diagnosed from the presence of frass (faecal pellets) extruded from the damaged host tissue. In some instances the infested stem also appears swollen and/or split, for example, with sweet potato borer damage. Another type of stem damage frequently seen results from insects feeding on the outer or epidermal stem tissue or even the bark of some trees.

3.4 Flower/Fruit Damage

Although many insects may utilise the flower to feed, any damage is not easily diagnosed since injured flowers will usually fall from the plant.

One may see at times though feeding lesions on the petals of some flowers prior to flower drop. Feeding marks from thrips may be observed on pigeon pea flowers, for example.

Much more apparent is damage to fruits. In this regard growers are very intolerant of any kind of pest damage which in fact is simply a reflection of the consumers' preference for unblemished fruit. Many insects may cause damage to fruit but again it may not be always easy to diagnose the causal agent.

Different types of damage may be broadly classified as:

3.4.1 Superficial damage is essentially cosmetic affecting only the appearance of the fruit without causing any internal injury, particularly in those fruit with a thick skin which is not consumed. Such damage, however, may allow for secondary infestation by pathogenic organisms leading to fruit rot. Even in the absence of secondary infestation superficial damage must be considered to be economic especially where fruit is targeted for the international market where the requirement is even more stringent for unblemished produce.

Some of the major pests causing this kind of damage include hemipteran insects (viz. scales, mealy bugs, white flies, coreid bugs, stink bugs etc.); thrips; some beetles and mites. Damage is due both from feeding as well as oviposition punctures. Very often superficial damage is observed as a scarring of the fruit due to 'corky' growths on the skin; malformed fruit may also result. Mites and thrips are usually responsible.

Banana is a very good example of a fruit which may show various types of superficial damage. Thrips may cause scarring by feeding and as a result of ovipositing in the skin tiny raised bumps are formed causing 'pimpling'. Fruit scarring beetles *Colaspis* sp. also cause damage. Very severe and characteristic scarring and fruit malformation of coconuts are caused by the coconut mite, *Eriophyes guerronis* Keifer. Here the damage may be quite pronounced with a large percentage of the external surface of the fruit deeply rutted with corky scabs extending from the calyx region where the fruit is attached to the bunch, down to its distal end. Oviposition marks are also frequently seen as dark spots on soft fruit, e.g. guavas, caused by fruit flies.

Sometimes superficial damage is a reflection of internal damage. Larvae of the Soursop moth, *Cerconota anonella* (Sepp) tunnel and feed in the pulp of the soursop fruit. After the adult has emerged from the fruit the tissue around the exit holes shows abnormal growth; hardened corky areas are formed and the fruit is often twisted.

3.4.2 Internal damage has a much greater impact on the crop since attacked fruit are rendered unfit for consumption as a result. Hemipteran insects are again an important group in this regard since their method of feeding results in both direct and indirect damage as described above for leaf damage. In this case since fruit represents a much better medium than leaf tissue, secondary infection by pathogenic organisms has a much greater impact leading to faster and more widespread rotting. Other insects with chewing and biting mouthparts consume significant portions of fruit and cause serious losses. Some of these like the tomato worm and the corn ear worm are borers.

One of the most widespread and destructive of fruit pests are the tephritid fruit flies. Tiny maggots from eggs laid in the skin make their way into the flesh of the fruit burrowing and eating their way inside. The flesh becomes mushy and unfit for eating. The presence of tiny oviposition punctures is a useful diagnostic character of some infested fruit. Usually, however, such punctures are not easily visible. Hence there is no external sign of damage. It is only on opening the fruit that the maggots or "worms" are visible. Other fly and beetle larvae may cause similar damage and may be secondary pests.

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5. GLOSSARY

ACOUSHI ANT: See Leaf-cutting ant.

BACHAC: See Leaf-cutting ant

BIOLOGICAL CONTROL: The utilization of natural enemies (parasites or predators) to control a pest population.

BLIGHT: A condition where the leaves of a plant go brown as a result of feeding by insects with piercing and sucking mouthparts.

BORER: An insect which eats and tunnels its way through plant tissue; usually larvae of lepidoptera, diptera and coleoptera.

CULTURAL CONTROL: The use of agronomic techniques for the control of plant pests.

DIE-BACK: Death of the plant, or part of the plant, resulting from pest attack to the underground parts.

ECONOMIC PEST: A species which impacts so adversely on a crop so as to cause an economic loss to the grower.

FRASS: Cellulose-rich faecal pellets produced by insects with biting and chewing mouthparts, particularly caterpillars.

GALL: Abnormal tissue development caused by insect and mite secretions, usually produced by actively growing leaf tissue.

HONEY DEW: Excess plant (phloem) sap which has been ingested by homopteran insects and excreted largely unaltered.

LEAF-CUTTING ANT: Hymenoptera, Formicidae, Attini; also called fungus-growing ants. Two main genera *Atta*, *Acromyrmex*. Individuals cut leaves and flowers which are used to grow a food fungus in an underground nest.

LEAF MINER: Insect larvae which feed on the mesophyll tissue of leaves leaving the epidermis intact. Larvae feed in a characteristic pattern which is observed as serpentine tunnels or leaf blotches.

LEAF ROLLER: An insect larvae which rolls a leaf to form a shelter inside of which it lives.

NATURAL ENEMY: An organism which attacks another in the field (see Biological Control).

OVIPOSITION: To lay eggs.

PHYTOPHAGOUS: Feeding on plants.

SCARRING: Plant damage resulting from mites or insects feeding by scraping the outer surface of fruits particularly. The plant reacts by producing corky tissue.

SHOT-HOLE: Leaf damage seen as numerous, small holes scattered over the leaf surface.

SOOTY MOLD: A black fungal growth on plant surfaces as a result of honeydew secretions by sucking insects.

STERILE INSECT TECHNIQUE: A pest control technique whereby males are sterilised by irradiation or by chemosterilants so that any mating with such individuals would result in non-viable egg production by females.

TOXICOGENIC: Poisonous or producing toxic effects.

DIAGNOSIS OF DISEASES OF FOOD CROPS CAUSED BY FUNGI

Fritz Elango

CONTENTS

DIAGNOSIS OF DISEASES OF FOOD CROPS CAUSED BY FUNGI

by

Fritz Elango

1. INTRODUCTION

Rapid and accurate diagnosis of plant disease and its cause is accepted as a pre-requisite for planning timely and rational control measures. Failure to diagnose properly and rapidly may result in crop loss, or in unnecessary use of pesticides with its attendant dangers to man and environment. Wrong diagnosis often leads to wrong control recommendations which sometimes worsen the disease situation. Iatrogenic disease is the term used to describe a crop disease which is induced or worsened by a plant pathologist's inaccurate diagnosis and prescription. Therefore, in an attempt to make plant protection more efficient and economical, correct diagnosis is emphasized.

2. PRINCIPLES OF DISEASE DIAGNOSIS

Diagnosis is recognizing the identity of a disease and this art is based on the scientific principle that a given plant responds pathologically and characteristically to attack by a given pathogen. Initial diagnosis depends on recognition of symptoms which in turn often depends on the appearance of diagnostic structures of plant pathogens termed signs. Therefore, knowledge of pathogen effects (symptoms) is important in diagnosis.

3. SAMPLE COLLECTION

Successful diagnosis depends on the collection of appropriate diseased specimens. The following guidelines are helpful in sample collection:

a. Collect fresh samples showing young as well as older stages of the disease

b. A healthy plant sample of the same variety and age for comparison with the diseased sample is helpful

c. If the cause of the disease is thought to be soil-borne, obtain the root system preferable with soil around it along with the entire plant

d. Package the specimen in a tight plastic bag if several days of storage without refrigeration is anticipated

e. Photograph of diseased plant is helpful in diagnosis

f. The sample should be sent to a diagnostic lab within the shortest space of time

g. The person submitting the sample should fill out a clinic diagnostic check list which includes all information on the crop which may be helpful in diagnosis (see sample check list on page ٢٨)

h. The disease diagnostic laboratory should acknowledge receipt of the diseased sample and inform the farmer or grower when results would be forthcoming or, if the sample is unsatisfactory, the diagnostician should request another. Such an acknowledgement reassures the grower that something is being done. This measure is also a good public relations exercise (see specimen acknowledgement on page ٢٩)

4. USEFUL EQUIPMENT FOR DISEASE DIAGNOSIS

1. Camera
2. Plastic/sandwich bags
3. Trowel
4. Magnifying glass 10X
5. Pocket knife/razor blade
6. Stereoscopic/compound microscope
7. Autoclave/sterilizer
8. Slides, needles, alcohol lamp
9. Glassware
10. Agar media reagents
11. General and specialized monographs

5. PRECAUTIONARY STEPS IN DIAGNOSIS

1. There are often difficulties in determining the cause of a disease because biotic and abiotic agents may cause similar symptoms. More than one pathogen may be involved e.g. wet rot of roots could be due to fungal and/or bacterial pathogens. Similarly, wilting may be due to water stress, *Pseudomonas solanacearum*, or *Fusarium oxysporum*.

2. For diagnosis, it is helpful to narrow the areas of confusion by being acquainted with:

   a. The common diseases of the host in question

PLANT DISEASE IDENTIFICATION FORM

Submit specimens to: EXTENSION PLANT PATHOLOGY
DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF ARIZONA
TUCSON, ARIZONA 85721

☐ CA ☐ C ☐ GA
☐ N ☐ HG ☐

Date collected _____

P.C. # _____

HOW TO COLLECT AND SHIP PLANT SPECIMENS FOR DISEASE DIAGNOSIS

The accurate diagnosis of a plant disease depends on receiving a fresh sample. All specimens should be fresh when collected, placed in a plastic sack and shipped immediately in a mailing tube or strong cardboard carton to prevent crushing enroute. When specimens arrive unidentified, wilted, crushed or in advanced stages of decay, diagnosis is often impossible. If the sample is in good condition, the disease can be diagnosed more rapidly. Most specimens will be diagnosed and acknowledged within a few days. Proper completion of this form will aid in prompt, accurate service.

GENERAL INFORMATION: Telephone Number _____

Owner or grower _____ Address _____
Collector _____ Address _____ Zip Code _____
Plant (or crop) attacked _____ Variety _____ Acreage _____
Plant part injured: Roots _____; Stem or branch _____; leaves _____; Flower _____; Fruit _____
General appearance: Wilted _____; dead areas _____; yellowed _____; stunted _____;
abnormal growth _____; leaf spot or blight _____; leaf mottle _____;
other _____
Distribution of diseases: Scattered plant _____; groups of plants _____; most of field _____;
on slopes _____; low ares _____; upland areas _____
When were symptoms first noticed? _____
Weather conditions of previous week: _____
Cropping history: _____
Chemicals applied, rates and date: _____
Fertilizer
Fungicide
Herbicide
Insecticide
Other
Location in relation to buildings: _____

ADDITIONAL INFORMATION FOR SPECIAL CROPS AND ORNAMENTALS:

Trees and Shrubs
Year established _____ Approximately age and size _____
Location: street or terrace _____; landscape _____; flower bed _____
Depth to caliche layer: _____
Shaded area _____; relation of injury to wind and sun _____
What is the watering schedule? _____

DIAGNOSIS AND PREVENTION:

Date reply: _____ L: _____ OC: _____ T _____ By _____

Reproduced from Streets (1972)

Ministry of Natural Resources

Plant disease clinic

Specimen Acknowledgement No. _____

The plant disease diagnosis laboratory is in receipt of a _____ plant specimen which arrived at the clinic in (excellent, fair, poor) condition.

☐ It is necessary for us to (culture, isolate, identify the pathogen from the submitted specimen.

☐ Diagnostic results will be forthcoming in approximately _____ weeks.

☐ Please submit another specimen, including roots ☐, leaves ☐, stem ☐, soil ☐

_____

Diagnostician

Date: _____

Adapted from Streets (1972)

b. Diseases peculiar to the area

c. Diseases expected at a particular season under given environmental conditions

3. The diagnostician must recognise an abnormal plant and be able to recognize signs and symptoms

6. SYMPTOMS CAUSED BY FUNGI ON PLANTS

In general, fungi induce local or general symptoms on their hosts. They cause three types of symptoms:

a. Necrosis (destruction of tissues) e.g.

- wilt = destruction of the vascular tissues e.g. *Fusarium*
- leafspot = destruction of the lamina e.g. *Alternaria*
- canker = destruction of the parenchyma e.g. *Colletotrichum*

b. Hypoplasia = stunting of plant organs or entire plant resulting from reduction in cell number and cell size

c. Hyperplasia = excessive growth of plant parts or whole plant resulting from increase in cell numbers and cell size (e.g. Witches' brooms disease of cocoa *Crinipellis pernisiosa* causes profuse, upward branching of twigs)

Symptoms often occur separately on different hosts, concurrently or in succession on the same host. Almost all the three types of symptoms described above also induce stunting of infected plants.

Common fungal symptoms (Figure 1) include the following:

Anthracnose = a necrotic and sunken ulcer-like lesion on the stem, leaf, fruit or flowers - of a plant. The causal fungus produces its spores in an acervulus

Blight = general and extremely rapid browning of leaves, branches, twigs and floral organs resulting in their death

Leafspot = localized lesions on host leaves consisting of dead and collapsed cells

Damping-off = rapid destruction and collapse of seedling plants near the soil level due to cortical decay

Canker = a localized wound or necrotic lesion, often sunken beneath the surface of the stem of a woody plant

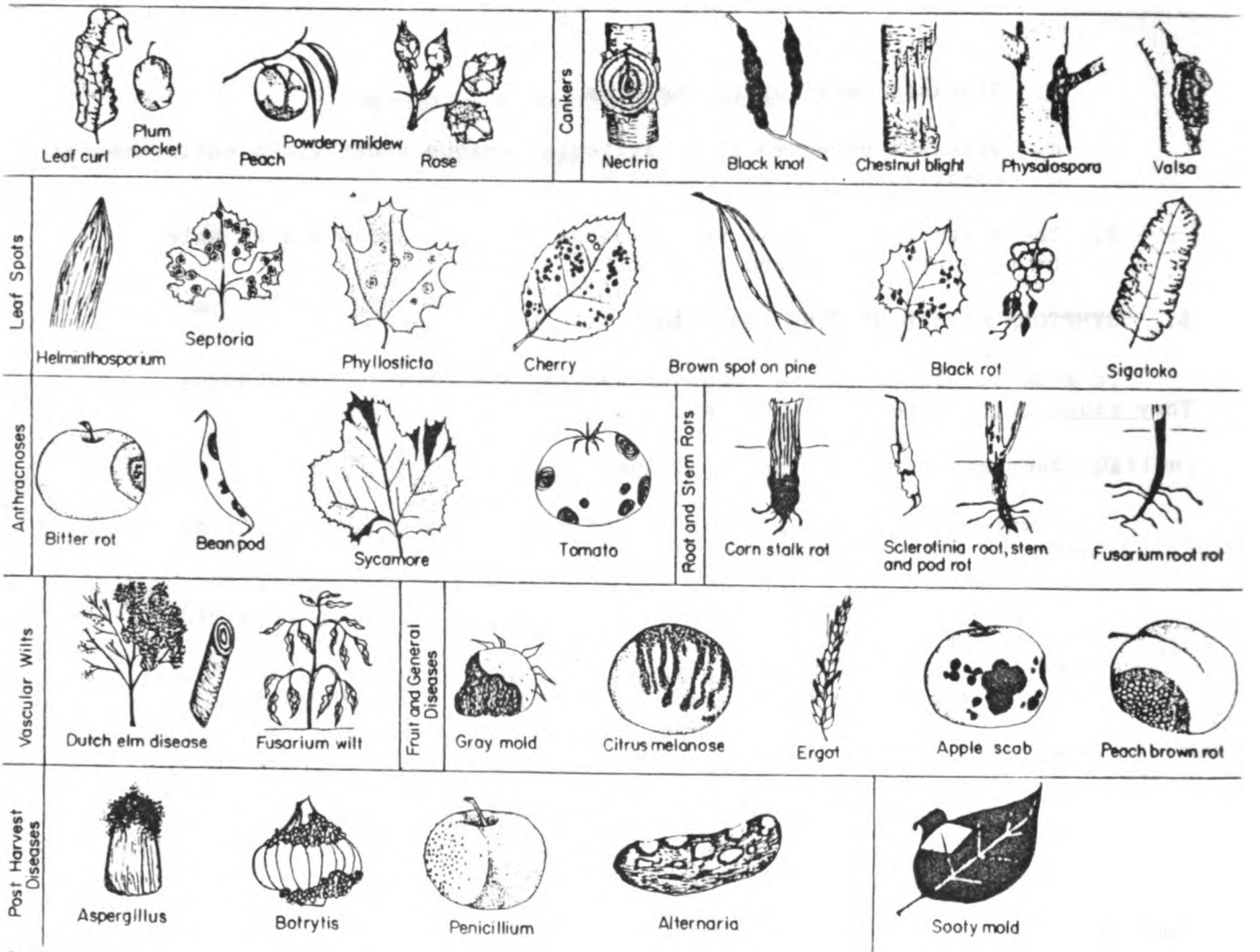


FIGURE 2
Common symptoms caused by some important Ascomycetes and Imperfect Fungi

Rust	=	many small rusty coloured lesions on leaves or stem
Root rot	=	decay of part or all of the root system
Soft rots/ dry rots	=	maceration and disintegration of fruits, roots, tubers and fleshy leaves
Wilt	=	a generalized secondary symptom in which leaves or shoots lose their turgidity and droop because of a disturbance in the vascular system due to insufficient water or toxic metabolites

In many diseases, the pathogen produces fruiting structures (signs) on the surface of the host. These include mycelium, sclerotia, pycnidia, acervuli, conidia, chlamydospores, etc. (see Fig. 2).

7. IDENTIFICATION OF FUNGAL PATHOGENS

1. There is usually no single or simple way of identifying fungi or diseases they cause

2. Identification is usually easier when disease occurs on an important economic crop because of the more readily accessible descriptions of pathogens and diseases.

3. Accurate identification of a disease requires identification of the pathogens, unless the disease is so well known that its causal agent can be determined from symptoms. In this case, much can be learned quickly by using pictures and illustrations. In this less scientific approach, visual comparison should be followed by referring to the description of the fungus and to the symptoms of the disease that it causes.

4. Before identifying the pathogen, first of all, identify the host plant by its technical (latin) name. Then read about pathogens associated with that host in a host index e.g. index of plant diseases in the American tropics. This short cut may result in tentative identification.

5. Where typical symptoms are present the fungus can be diagnosed by using a hand lens, magnifying glass or stereoscopic microscope to check for fruiting structures (Fig. 1). If fruiting structures are associated with diseased tissues, find answers to the following questions:

   a. Is mycelium septate or aseptate?

   b. What kind of reproductive structures are produced?

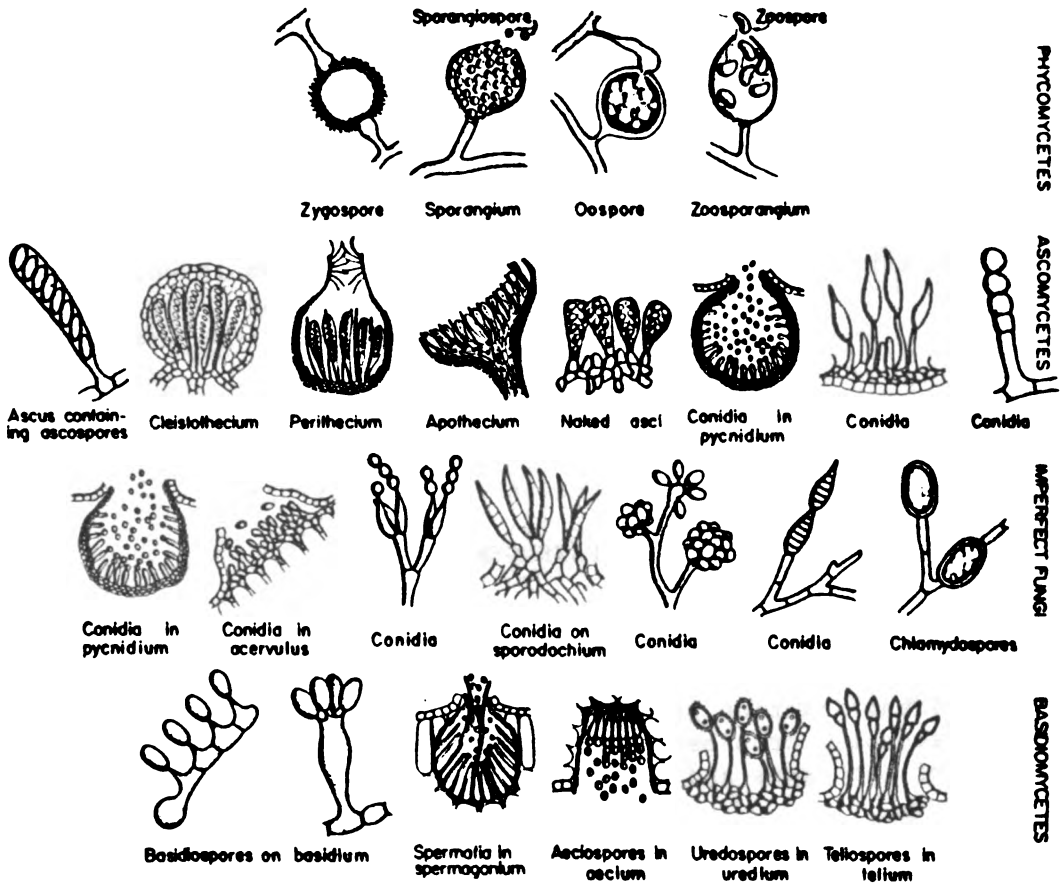


FIGURE 2.
Representative spores and fruiting bodies of the main groups of fungi.

(i) sporangia, zygospores, antheridia, oogonia, oospores (Phycomycetes)

(ii) conidia on free sporophores (Moniliales)

(iii) conidia with pycnidia (Sphaeropsidales)

(iv) conidia in acervuli (Melanconiales)

(v) Ascocarp (where spores are borne in asci cleistothecium, perithecium and apothecium)

(vi) Basidiocarp (Basidiospores borne on basidia)

(vii) Spore morphology (colour, size, shape, conidial ontogeny)

Using these characteristics, key or trace the pathogen to genus and species using identification keys/specialised monographs e.g. Barnett, Alexopoulos, Von Arx, etc. For example, if conidia are produced in pycnidia start keying in the Sphaeropsiddles or check host index for pathogens belonging to this order. If no pathogen signs are available, do a detailed examination of the diseased sample for additional symptoms and look for the pathogen inside the diseased plant. Using a knife, cut a sample from the plant and examine the vascular tissues, roots, base of the plant etc. If this fails to give any clue, do two things: incubate the tissues in a humid chamber to induce sporulation and do a routine isolation of the causal agent(s) on an appropriate laboratory medium. Incubate until fungus sporulates to allow for identification.

6. Determine whether the observed fungus is a pathogen or saprophyte by studying the morphology of its mycelium, fruiting structures and spores and by consulting appropriate books of plant pathology to see whether the fungus has been reported to be pathogenic on the host on which it was found. This may be confirmed by pathogenicity tests.

7. If the symptoms caused on the host correspond to those given as caused by that particular fungus, then the diagnosis is considered complete. However, if no such fungus causes symptoms on the host under study, the fungus could be considered a saprophyte while the search for the causal agent continues. If, however, the pathogen found appears to be the cause of the disease but no previous reports support this, then Koch's rules of proof of pathogenicity of that fungus must be demonstrated in order to prove that the isolated pathogen is indeed responsible for the disease. Koch's rules are as follows:

   1. The organism must be found constantly in association with the diseased sample

2. The organism must be isolated and grown in pure culture and its characteristics described

3. The organism from pure culture must be artificially inoculated on healthy plants of the same species or variety on which the disease appears, and it must produce the same disease symptoms

4. The organism must be re-isolated in pure culture and its characteristics must correspond exactly like those observed in step (2) of these rules

8. CONTROL OF FUNGAL DISEASES OF PLANTS

Having diagnosed the disease and identified the causal fungus to genus and species, the next question is: how can the disease be controlled? Disease diagnosis is meaningless to the farmer if not accompanied by control recommendations. Where does one find recommendations for the control of fungal pathogen? These can be found in appropriate books of plant pathology. However, in making control recommendations, the diagnostician must also find answers to the following questions:

a. Is the disease incidence on the plant(s) enough to warrant control?

b. Can the grower afford to carry out the control recommended? Are there cheaper and more practical alternatives?

c. Does the disease require immediate treatments or a long-term control measure(s)?

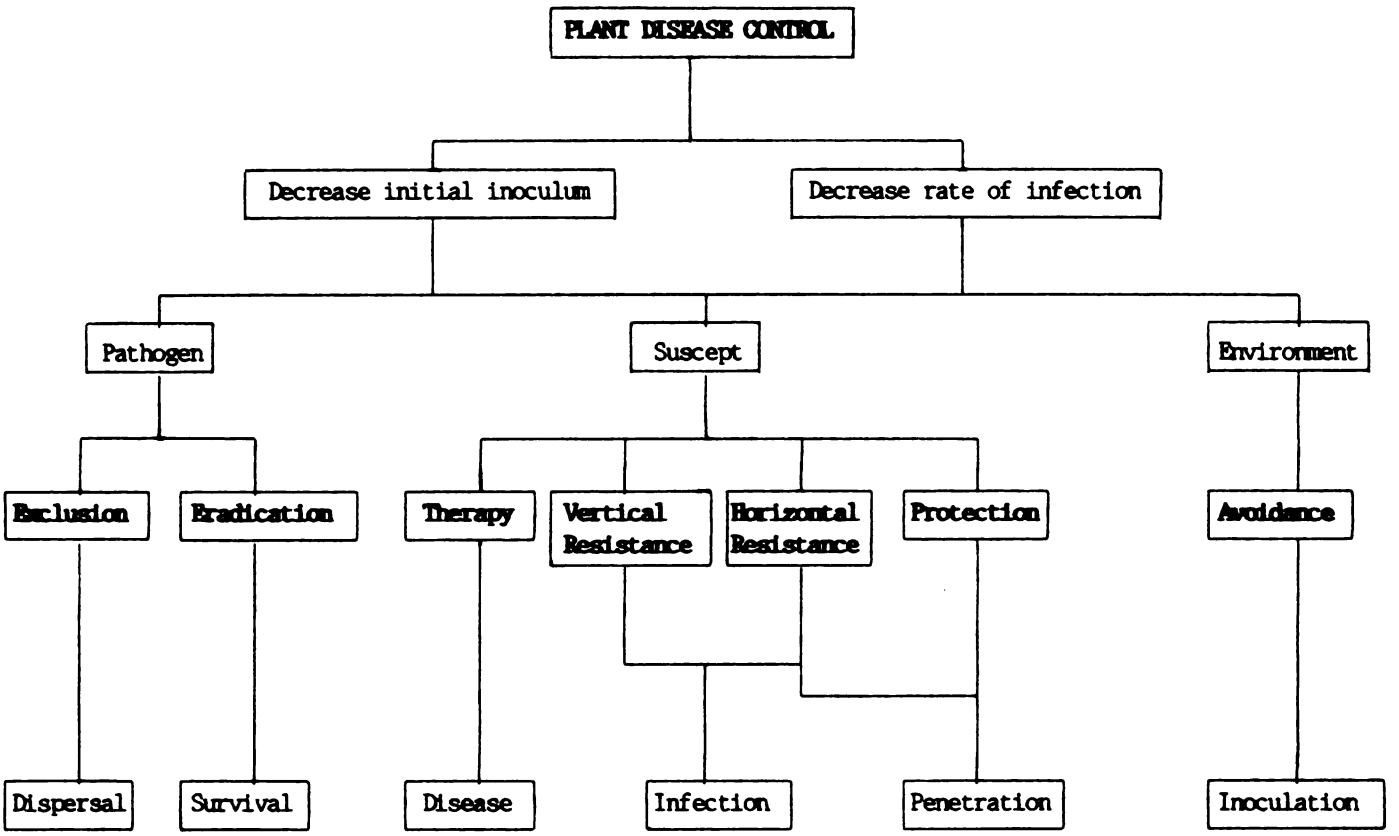
d. Is the disease likely to reoccur?

e. How does the pathogen survive and for how long?

f. What are the possible sources of inoculum and how is the pathogen disseminated?

However, given the variety and complexity of fungal diseases, a large number of control measures have been developed, and these take into consideration the characteristics and life cycle of each fungus, its habitat and environmental preferences. Generally, a combination of measures (integrated control) offers better disease control than a single one.

The principles of plant disease control shown on the following flow chart apply to fungal pathogens as well.



Interrelations between the principles of plant-disease control, epidemiological approaches to disease control, the objects or "targets" of disease-control measures, and events in the life cycles of plant pathogens interrupted by the control measure. (After Roberts & Boothroyd, 1984)*.

Specific controls for fungi include the following:

1. Use of pathogen-free seed or propagating stock

2. Destruction of plant parts or refuse harbouring the pathogen

3. Destruction of volunteer plants, alternate/alternative hosts

4. Use of clean tools, containers, proper drainage of fields and aeration of plant - cultural practices

5. Crop rotation is satisfactory for some fungal pathogens but not for those that have a wide host range or whose resting structures are long-lived

6. Use of resistant varieties - promising and attractive because of its low overall cost

7. Most effective control and sometimes the only control measure is chemical sprays/dust to the plant, seed or in soil where the plants are to grow (using contact or systemic fungicides). For high value cropped soils, sometimes soil-borne fungi are controlled by steam or electric heat or by volatile liquids e.g. methyl bromide.

8. Biological control

9. PRACTICAL EXERCISE FOR THE DIAGNOSIS OF FUNGI

9.1 Introduction

Plant pathogenic fungi can be taxonomically classified into four groups whose characteristic fruiting structures (signs) have been given in Figure 2 of the lecture notes. Diseased specimen from these four groups shown below will be diagnosed according to the procedure given in the lecture. Healthy control plants are provided. Most of the diseased samples have also been classified on the basis of symtoms induced on host plants. Given the time limitation, each diseased specimen has been induced to sporulate and both a prepared slide and a pure culture have been made for you to examine under the compound microscope. A few modifications in the diagnosis of vascular wilts and seed-borne diseases have been given for your guidance. Use the general and specialized monographs as much as possible. The Instructor and demonstrators would be pleased to answer any questions.

9.2 Classification on the basis of causal agents

(i) Phycomycetes
- e.g. Pythium root rot of tannia *(Xanthosoma sagittifolium)*
- e.g. Phytophthora pod rot of cocoa *(Theobroma cacao)*

(ii) Ascomycetes
- e.g. Powdery mildew of cowpeas *(Vigna unguiculata)*
- e.g. Sigatoka disease of banana caused by *Mycosphaerella musicola*

(iii) Basidiomycete
- e.g. Rust of pigeon peas *(Cajanus cajan)*
- e.g. Smut of corn *(Zea mays)*

(iv) Imperfect fungi
- e.g. Fusarium wilt of eggplant *(Solanum melongena)* (Conidia in Sporodochia) - Moniliales
- e.g. Septoria leafspot of passion fruit *(Passiflora edulis)* (conidia in Pycnidia) - Sphaeropsidales
- e.g. Yam anthracnose disease (conidia in Acervuli) - Melanconiales

9.3 Classification on the basis of symptoms

a. Leafspot diseases e.g. banana or Septoria leafspot

b. Vascular wilts e.g. Fusarium wilt of eggplant

c. Soil-borne/root rot diseases e.g. Pythium root rot of tannia

d. Postharvest diseases e.g. *Botryodiplodia, Colletotrichum*

e. Seedborne diseases e.g. Diseases of bean

9.4 Diagnosis of vascular wilt diseases

To induce sporulation in vascular wilts, make a cross-section of the stem, a few centimetres above the soil level. Incubate a few thin disc sections on moist filter paper before isolating and identifying the pathogen.

9.5 Seed-borne diseases caused by fungi

Many parasitic fungi reduce seed yields both qualitatively and quantitatively. Saprophytic fungi also lower seed quality through seed discoloration. The following symptoms are associated with fungal infection of seed.

a. Seed abortion e.g. corn smut

b. Shrunken seed, reduced seed size

c. Seed rot

d. Sclerotisation of seed

e. Seed necrosis

f. Seed discoloration e.g. Fusarium infection

g. Reduction or elimination of germinaiton capacity, lowered viability

h. Physiological alterations in seed e.g. mycotoxin caused albinism and virescence

9.6 Procedure for diagnosis of seed-borne fungi

1. Sampling followed by surface sterilization

2. The blotter test] incubate under appropriate temperature
3. The agar plate test] and dark or light conditions to induce sporulation

4. Seedling symptom test

5. Fungal identification

6. Control recommendations:
   - Fungicidal seed treatments
   - Hot water treatment (pre-soaking plus 50°C treatment for definite period of time)
   - Dry heat treatment

9.7 General and specialized monographs provided

- CMI Descriptions of Pathogenic fungi and bacteria
- Plant pathology - AGRIOS (1978)
- Introductory Mycology - Alexopoulos & Mims 1979
- Identifying Diseases of Vegetables - Penn State Univ. 1983
- PANS Manual Vols I - IV
- Diseases of the American Tropics - Wellman
- Identification of Imperfect Fungi - Barnett 1960
- The Diagnosis of Plant Diseases - Streets 1972
- Fungal Diseases of Tropical Crops - Holliday (1984)
- Fusarium species - CMI

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DIAGNOSIS OF PLANT VIRUSES AS AN AID TO DISEASE CONTROL

Syed Q. Haque

CONTENTS

DIAGNOSIS OF PLANT VIRUSES AS AN AID TO DISEASE CONTROL

by

Syed Q. Haque

1. INTRODUCTION

Identification of plant viruses involve highly sophisticated laboratory and glasshouse investigations by Scientists. Knowledge derived from such investigations provide information for the field diagnosis of virus diseases by farmers and Extension staff.

Investigations carried out by the Regional Research Centre of the University of the West Indies, and the Caribbean Agricultural Research and Development Institute, over the last 15 years have provided information for the field diagnosis of important virus diseases of crop plants with respect to territories of the Commonwealth Caribbean.

Most viruses produce visible symptoms on the plants they infect, although some virus-host combination may not produce visible symptoms. Many of the viruses produce diagnostic symptoms in a particular host, although there are instances where widely different viruses produce similar or overlapping symptoms on the same plant species. On the other hand, a particular virus may produce different symptoms in different host plants and even in different varieties of the same plant species.

Fortunately, in the Caribbean, we have much fewer virus diseases of economic importance although we grow a fairly wide range of crop plants and our growing season is year round. Several crop plants do not suffer from any virus disease, although elsewhere, they are devastated by one or the other virus disease. A farmer or an Extension staff will, therefore, need to come to grips with a manageable list of virus diseases.

A sensitive aid to diagnosis of plant viruses is the use of antisera. Antisera tests can be performed even in the field with simple tools quickly. Wherever feasible and necessary, such tests should be performed.

A knowledge of which virus diseases to expect on which crop and at what stage of the development of the crop is important. Familiarity with the development of the disease - when and where it begins, its course, and

how fast it spreads - is critical to diagnosis as an aid to control. The control of virus disease should preferably be proactive rather than reactive. If it has to be reactive, it should to be as early as possible.

Several symptoms of non-viral origin may be confused with those caused by viruses, unless carefully diagnosed. Common examples are mite damage on cassava, herbicide (2,4-D) damage on tomato and nitrogen deficiency in papaya. A non-virus symptom is not transmissible. Generally speaking, nutrient deficiency and toxicity, herbicide damage and drought conditions may cause virus-like symptoms. Certain pesticides and even mineral oil carriers, particularly in stronger doses, could cause toxicity. These possible situations must be examined in establishing diagnosis of virus diseases.

2. IMPORTANT PLANT VIRUS DISEASES OF THE CARICOM REGION

Crops and Diseases	Parts affected					Transmission								Control and Remarks
	Leaves	Branches/stem	Fruits	Tubers	Roots	Seed	Tuber	Stem cutting	Budwood	Plant debris	Tools	Worker's hand	Aphid	Leaf Hopper	Beetle	Whitefly	
ROOT CROPS																	
Yam																	
Internal Brown Spot (IBS) and Mosaic	* X			X				X			X						All five species of yams (*Dioscorea alata*, *D. rotundata*, *D. cayenensis*, *D. trifida* and *D. esculenta*) affected by mosaic. Only *D. alata* by IBS. Use virus-tested tubers for planting or tubers from symptom - free plants
Tania, Eddoe & Dasheen																	
Dasheen Mosaic virus	X			X				X			X		X				Use planting material from disease-free plants. Control aphids. Roguing
Cassava																	
Cassava Common Mosaic virus	X	X						X									Use cuttings from disease-free plants. Disease not widespread yet, but needs to be watched.
FOOD LEGUMES AND CEREALS																	
cowpea																	
Cowpea Severe Mosaic virus	X	X	X			X									X		Use resistant variety or disease-free seed. Rogue early infection. Control beetle. The incidence of virus is less in dry season

*X indicates positive role

Crops and Diseases	Parts affected					Transmission									Control and Remarks
	Leaves	Branches/stem	Fruits	Tubers	Roots	Seed	Tuber	Stem cutting	Budwood	Plant debris	Tools	Worker's hand	Aphid	Leaf Hopper	Beetle	Whitefly	
Cucumber Cucumber mosaic (CMV)	X		X			X							X				Use disease-free seed. Practice early roguing. Control aphids.
Lettuce Lettuce mosaic	X					X							X				Incidence very low. Use disease-free seed.
Eggplant Eggplant mosaic															X		Widespread but local varieties are tolerant. Beetle vector is also a common pest of eggplant. Economic losses low. Control beetles if necessary.
FRUITS **Papaya** Bunchy top (mycoplasma disease)	X	X	X											X			Discard affected seedlings. Decapitate affected leaves and stem. Control leaf hoppers
Papaya Ringspot	X	X	X										X				Discard affected seedling. Decapitate affected leaves and stem. Control aphids.

Crops and Diseases	Parts affected					Transmission									Control and Remarks
	Leaves	Branches/stem	Fruits	Tubers	Roots	Seed	Tuber	Stem cutting	Budwood	Plant debris	Tools	Worker's hand	Aphid	Leaf Hopper	Beetle	Whitefly	
Dry bean																	
Bean common Mosaic	X		X			X							X				Use resistant varieties. Use disease-free seed. Control aphids. Seed transmission very high.
Golden Mosaic	X		X			X										X	Use resistant varieties. Use disease-free seed. Practice crop rotation. Control white flies. Disease important in Jamaica only.
Corn																	
Maize Mosaic	X	X												X			Causes stunting of plants. Stripes and mosaic on leaves. Early roguing of disease plants. Incidence generally low. Control leaf hopper when incidence is high
VEGETABLES																	
Tomato																	
Tomato mosaic (TMV)	X	X	X			X			X	X	X						Use resistant varieties. Use disease-free seed. Remove debris. Practice sanitation in handling plants. Discard affected seedling.
Pepper (hot, sweet)																	
Pepper mosaic (PVY)	X	X	X										X				Use resistant varieties. Practice early roguing and early control of aphids. Economic losses higher in sweet pepper. Low to negligible in hot pepper.

Crops and Diseases	Parts affected					Transmission									Control and Remarks
	Leaves	Branches/stem	Fruits	Tubers	Roots	Seed	Tuber	Stem cutting	Budwood	Plant debris	Tools	Worker's hand	Aphid	Leaf Hopper	Beetle	Whitefly	
Citrus																	
Exocortis	X	X				X			X		X						Use of certified budwood and resistant root-stock
Tristeza		X			X				X				X				Use of certified budwood and resistant root-stock
Psorosis	X	X				X			X		X						Use of certified budwood

3. CONTROL OF VIRUS DISEASES OF PLANTS

Prevention rather than cure is the best method for the control of plant diseases. This is all the more true in the case of virus disease since effective curative measures are not available.

Before planning effective control measures it is essential to know:

(i) The identity of the virus: Different viruses may produce similar symptoms on the same plant (e.g. Lettuce mosaic virus and Tomato spotted wilt virus on lettuce; the former being seed-borne). A virus may be transmissible by sap only (tobacco mosaic virus) or only by insect vectors (Papaya ringspot virus) or by both (Cowpea Severe mosaic virus). The viruses of the vegetatively propagated plants are generally carried in the planting material, or through vegetative parts used in propagation.

(ii) The identity of the vector: The majority of the vectors are insects, particularly aphids, white flies and leaf hoppers. But some virus diseases may be transmitted by mites (e.g. Fig mosaic virus by *Aceria fieus*), nematodes (e.g. Grape vine fan-leaf by *Xiphinema index*) and even fungi (e.g. Tobacco necrosis virus by *Olpidium* spp.).

(iii) The source of the virus and vector: The initial source of infection may be from within the crop in the seed, planting material, volunteer plants or the infected remains of the previous crop. It may be from outside the crop on certain alternative hosts including cultivated crops, weeds or wild plants. The vector also may be harbouring on these alternative host plants.

(iv) The transmission and the disease cycle: Usually the transmission cycle of the viruses transmitted by aphids is short consisting of several minutes. Most of the aphids lose the virus quickly. The viruses transmitted by white flies and leaf hoppers have longer transmission cycles extending to several hours or even several days. The white flies and leaf hoppers may remain infective for quite a long time.

Several other host plants, particularly weeds may be involved in the disease cycle of some virus diseases.

Based on the information available from the foregoing, the control measures may be planned by using a desirable combination of the following methods:

1. Use of virus-free seed and planting material

   a. Use of resistant varieties

b. Selection and use of disease-free seeds and planting materials

c. Use of certified disease-free seed and planting materials like tubers, rhizomes, cuttings, budwood material etc.

2. Clean cultivation:

a. Removal of infected remains of the crop

b. Removal of weeds in and around the crop which may harbour viruses

3. Roguing of the crop - Infected plants should be pulled out and then burnt or buried in a suitable place.

4. Crop rotation - Using such crops that are not infected by the particular virus.

5. Hygiene measures - Several viruses (e.g. tobacco mosaic virus, potato virus X) are spread by man by simultaneouly touching diseased and healthy plants. Care should be exercised in staking and tying tomato plants. All healthy plants should be tied first before handling virus affected plants. Brushing of worker's clothes from diseased to healthy plants should be avoided. Workers should wash hand with soap before and after handling plants.

6. Use of crop protection Chemicals:

Various pesticides may be used to kill and inhibit the activity of plant-virus vectors, but for various reasons pesticides have only been successfully used to control a few viruses. Pesticides seem to be most useful for controlling viruses that are acquired and transmitted by their vectors only during long feeds, and for viruses that are transmitted mainly within the crop instead of coming from outside. In planning spray programmes note must be taken of possible differences in the role of insects as vector of viruses and as pests of crops.

For virus disease transmitted by aphids quickly, emulsion of water and non-toxic oil (5 to 10% oil) has been found to be effective in reducing the incidence of virus diseases.

7. Conclusion:

While using a susceptible variety, viruses are difficult to control. The aim should be to reduce losses at economic costs. Farmers and Extension staff must, therefore, understand the type and extent of losses caused by various virus diseases. Control measures should be planned timely and intelligently.

THE DIAGNOSIS AND CONTROL OF FIELD PROBLEMS CAUSED BY BACTERIA

Cynthra Persad

CONTENTS

THE DIAGNOSIS AND CONTROL OF FIELD PROBLEMS CAUSED BY BACTERIA

by

Cynthra Persad

1. INTRODUCTION

Bacteria are unicellular organisms, are very small, their length varies between 1 - 3μ, and are usually visible under high magnification of a microscope.

1.1 Shape

Bacteria can be spherical or cocci, cylindrical, often called rods or bacilli or spiral or vibrio in shape. All plant pathogenic bacteria are rod-shaped.

1.2 Structure

The cell substance is covered by a cytoplasmic membrane which is surrounded by a more rigid cell wall, giving shape to the cell. Nutrition takes place across this membrane.

The cytoplasmic area is rich in RNA-ribosomes which contains many enzymes necessary for protein synthesis.

Since plant pathogenic bacteria do not form spores, the bacterial capsule or slime substance assumes greater importance. The capsule-enclosed bacteria are resistant to external influences (e.g. heat, light, chemical, etc.) and in this way maintain their viability.

Bacterial cells do not possess a distinct nucleus but the genetic material is arranged in a long strand called a genophore; 2μ long in a closed loop within the cytoplasm.

1.3 Motility

Most plant pathogenic bacteria are motile, and possess a flagellum (or flagella).

Flagella - these are extremely thin hair-like appendages (0.1μ wide) which are not visible under the light microscope but are apparent only with special staining procedures.

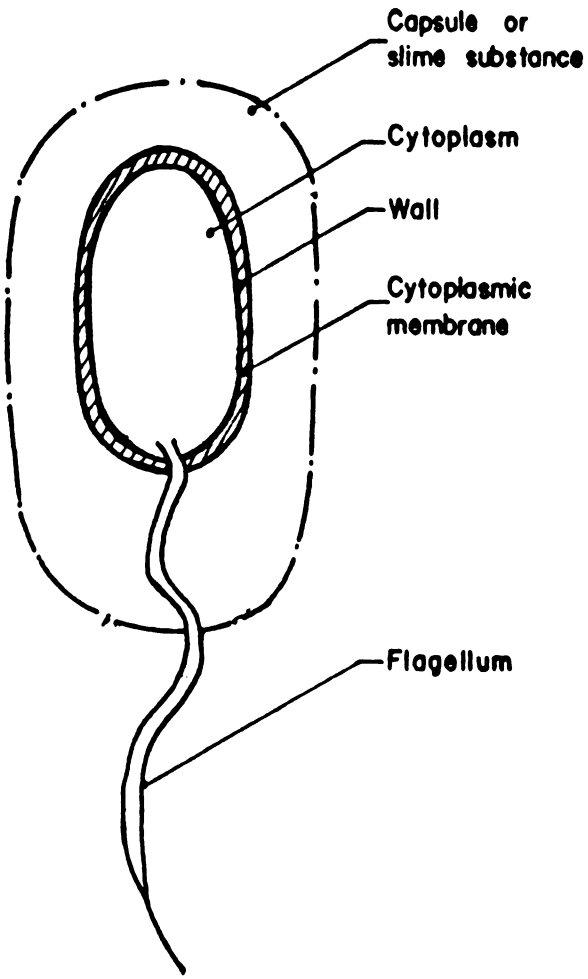


Fig. 1 Anatomic structure of a bacterium

The number and arrangement of the flagella are species variable and help to characterize the different bacterial genera.

1.4 Multiplication

Reproduction is mostly asexual by simple cell-division or fission forming colonies on solid media. The separation of two halves is not always complete, so that chains of two or more cells may be formed.

1.5 Temperature for Growth

The bacterial cell is able to survive low temperatures, and reproduction may begin as low as 5 - 10°C.

The optimum growth temperature is between 25 - 30°C. Plant pathogenic bacteria cease to reproduce between 33 - 40°C.

Rate of growth is influenced by the pH of the medium and bacteria develop well on neutral or slightly alkaline media (pH 7.0 - 7.2).

Bacteria can be classified according to the material from which they obtain their carbon and nitrogen requirements - e.g.

Autotrophs - Bacteria capable of building their cells from inorganic compound, e.g. nitrifying bacteria

Heterotrophs - Bacteria which require energy for vital processes obtained from decomposing organic material - most plant pathogenic bacteria are found in this group

Paratrophs - These are true obligate pathogens reproducing only in host tissue - (no plant pathogenic bacteria)

1.6 Anaerobes/Aerobes

Some bacteria will grow and multiply either in the presence or absence of air - these are known as facultative anaerobes.

Others remain dormant or are killed in the presence of air - obligate anaerobes.

Most plant pathogenic bacteria are aerobes, though some are facultative anaerobes.

1.7 Bacteria as Antigens

Bacteria injected into the blood-stream of an animal excite the formation of substances in the animal's blood-serum, antagonistic to the injected bacteria. These antagonistic substances produced are known as antibodies. Serum containing the appropriate antibodies when

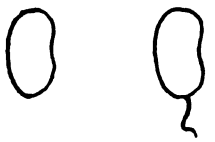
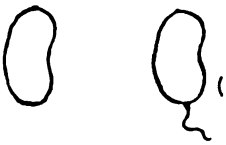
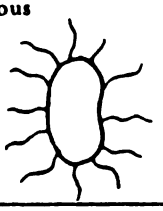
	Motility	Colony on nutrient broth agar	Gram stain	Type of Disease (with few exceptions)
Agro-bacterium	Motile or non-motile 	White	–	 Hypertrophy
Coryne-bacterium	Non-motile or motile  (2 spp)	Cream	+	 Wilt Vascular diseases
Erwinia	Peritrichous 	White	–	 Soft rot  Die-back
Pseudomanas	Lophotrichous  (More than one flagella)	Greyish white transparent and diffusable green flourescent pigment	–	 Leaf Spot  Die-back  Wilt
Xanthomonas	Monotrichous 	Yellow	–	 Leaf Spot

Fig. 2 Some morphological and pathological properties of plant pathogenic bacteria

Adapted from: Methods in Plant Pathology by Z. Kiraly, Z. Klement, F. Solymosy and J. Voros

b. with some exceptions e.g. rootknot nematode infection, determination of nematode populations, both qualitative and quantitative, is essential to an accurate diagnosis

c. the sampling techniques, handling and processing of samples are critical to arriving at a proper diagnosis.

4. SAMPLING

Sampling methodology will be determined by several factors. These are:

a. the type of crop to be sampled i.e. annual or tree crop;

b. the size of plot to be sampled;

c. the appearance of the field e.g. patchy growth;

d. fallow plots.

4.1 Crop Type

If the plants are small e.g. tomato, then samples are taken by removing the entire root system and collecting rhizosphere soil. For larger plants e.g. bananas, samples of soil and root should be taken in the region of feeder roots i.e. about 45 - 60cm from the pseudotrunk. It may be necessary to uproot the entire banana plant so that samples of the rhizome can be taken. For tree crops, samples of soil and root in the region of the feeder roots i.e. the 'drip-line', should be taken.

4.2 Plot size

For small plots, soil and root may be taken from about 3 - 5 plants. The larger the plots the greater the number of samples to be taken. These samples can be bulked to form a composite sample.

4.3 Field Appearance

When patchy responses by plants are recognised then separate samples are taken from plants showing "thrifty" and "unthrifty" growth. Where, however, there are no discernible differences in responses by plants, then random samples may be taken diagonally across the field.

4.4 Sampling of Fallow Plots

A soil augur is used to sample fallow plots. The depth of sampling depends on several factors including soil type and soil moisture content.

4.5 Bagging, Labelling and Protecting Samples

Place soil and root samples separately in polyethylene or similar moisture-retaining bags. Plastic labels with the following information

observed under the dissecting microscope as tiny longitudinal discoloured specks.

Lesions, however, may originate internally by the feeding and reproduction of endoparasites such as *Pratylenchus* spp. and *Radopholus* spp. Lesions arise in the cortex by the death of cortical cells and may increase in size and girdle small roots. Similar lesions may be observed in the rhizomes of plantains and bananas.

3.2.2 Root rot

The punctures created by the feeding activities of plant-parasitic nematodes are invaded by bacteria and fungi which promote the rot of the cortex followed by the stele. The soil moisture content influences greatly the rate at which this rot occurs.

3.2.3 Galls or Knots

Some groups of nematodes e.g. root knot nematodes stimulate increase in cell size e.i. hypertrophy and increase in cell number i.e. hyperplasia. The appearance of well developed knots in rootsystems facilitates a relatively easy diagnosis, although other nematodes may be contributing to the problem. It is important to recognize the difference between knots or galls and nodules in legumes.

3.2.4 Stubby-root

Sometimes, nematodes instead of stimulating cell activity, depress it. These nematodes e.g. *Trichodorus* spp. feed externally on growing tips, suppressing cell division and creating a stubby-root system. In some instances plants may tend to topple e.g. melongene.

3.2.5 Beard root system

Some nematodes stimulate the initiation and development of roots just above the point of invasion. This results in excessive root branching giving the system a 'bearded' appearance.

It is important to note that field examination of symptoms is critical to the diagnosis of a nematode problem. One must recognise, however, the following facts:

a. many of the symptoms induced by nematodes can also be induced by other agents both biotic and abiotic

corn and sweetpotato, respectively. In the Windward Islands, annual loss of banana, caused by plant-parasitic nematodes, is substantial.

3. NEMATODE-INDUCED SYMPTOMS

Symptoms induced by nematode infection may conveniently be divided into those that appear above-ground and those that exist below-ground. There is, however, a direct relationship between the above-ground and below-ground symptoms.

3.1 Above-ground Symptoms

3.1.1 Poor plant growth

The poor overall appearance of plants in a field or patches of plants in a field may indicate that a nematode problem exists. The injurious effects nematodes have on plants may result in stunting, chlorosis and poor yields. During periods of inadequate rainfall, wilt symptoms may appear.

3.1.2 Die-back

In perennials affected by nematodes e.g. citrus, symptoms similar to those caused by drought and malnutrition occur, leading to 'slow-decline'. Symptoms include die-back of twigs.

3.1.3 Necrosis, crinkling and twisting

Some nematode infections result in necrosis and distorted growth of shoot parts. *A. besseyi* infection of rice leads to discolouration of leaf tips which eventually turn necrotic. The flag leaf becomes crinkled and distorted. The size of the panicle and the number and size of the grains are reduced.

3.1.4 Discolouration

Infection of coconut by *R. cocophilus* leads to a red or orange-red ring of discoloured tissue about 3cm wide, 2.5cm under the stem surface. Discolouration of the trunk is accompanied by yellowing and then browning of the lower leaves.

3.2 Below-ground Symptoms

Development of symptoms in the rootsystem depends on the host response and the qualitative and quantitative components of nematode populations.

3.2.1 Lesions

The ectoparasites puncture the roots when they feed. The punctures may be termed primary lesions and are usually

DIAGNOSIS AND CONTROL OF NEMATODE PROBLEMS OF FOOD CROPS OF THE CARIBBEAN

by

George Bala

1. WHAT ARE NEMATODES?

Nematodes or round worms belong to the phylum Nematoda and are characterised by having relatively highly specialised development of the muscular, excretory, nervous and reproductive systems. They possess no circulatory or respiratory system. Members of the phylum display great variation in size, shape and habitat.

Generally, nematodes may be divided into the saprozoic or free-living and the parasitic forms. The latter group of nematodes may be further classified into animal and plant-parasitic nematodes.

The plant-parasitic nematodes comprise about 10% of the total nematode population and are distinguished from other groups of nematodes by the possession of a protrusible stylet. They are colourless and range in length from 0.3mm - 12.0mm. Of the 2,000 described species, the great majority belong to the Order Tylenchida and the remainder to the Superfamily Dorylaimoidea.

2. IMPORTANCE OF PLANT-PARASITIC NEMATODES

Plant-parasitic nematodes inflict severe damage on crops of agricultural importance. They cause physical injury to rootsystems impairing their ability to absorb water and minerals and simultaneously, affecting their anchorage function. Punctures on roots act as portals of entry for secondary pathogens. In some cases, infection of various parts of the shoot system may occur e.g. *Aphelenchoides besseyi* on rice and *Rhadinaphelenchus cocophilus* in coconut.

Generally, plant-parasitic nematodes are thought to cause, world-wide, an average of 10% loss in yield. A crop-loss survey in the United States of America in 1970 indicated that nematodes may cause average losses of 5-20%. Losses in yield in the region of 25% have been recorded for bean, carrot, cucumber and melon and 15% loss in yield has been reported for green pepper and tomato. In the Caribbean, preliminary data have shown losses in yield, due to nematodes, of 28%, 32%, 40% and 92% in tomato, soybean,

5. Nematode Extraction and Staining Techniques

5.1 Extraction of nematodes from soil

5.1.1 Direct Extraction using Modified Baermann's Funnel technique

5.1.2 Cobb's Gravity and Sieving and Modified Baermann's Funnel techniques

5.2 Extraction of Nematodes from Root

5.2.1 Maceration of roots

5.2.2 Maceration of roots, tubers, bulbs etc for quick diagnosis

5.3 Staining Roots

6. Processing Nematode Samples

6.1 Concentrating nematode suspension

6.2 Killing nematodes

6.3 Fixing nematodes

7. Identification of Nematodes

7.1 Examination under the dissecting microscope

7.2 Temporary slide preparation of nematodes

7.3 Picking nematodes

7.4 Examination under the light compound microscope

8. Counting Nematodes

8.1 Soil, root and rhizome nematode populations

8.2 Stained-root nematode populations

8.3 Rootknot nematode indexing

9. Nematode - Pathogen Interactions

10. The Diagnosis and Guidelines for Recommendation for Control

11. Steps in Diagnosis of two selected examples of Nematode Problems

11.1 Rootknot, reniform and other nematodes associated with tomato

11.2 Nematode problems of plantains and bananas

DIAGNOSIS AND CONTROL OF NEMATODE PROBLEMS OF FOOD CROPS OF THE CARIBBEAN

George Bala

CONTENTS

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RESULTS OF TESTS ON REPRESENTATIVE BACTERIA

TESTS	A	B	C	D	E	F	G
Colour							
Gramstain							
Kovac's Oxidase Test							
Fluorescence on KB							
Acid from Glucose							
Aerobic							
Anaerobic							
Soft rot of Potato							
Nitrate Reduction							
Starch Hydrolysis							

Details of these tests have been given in Appendix I together with a simplified diagnostic key useful in identifying plant pathogenic bacteria.

N.B. It will be necessary for students to work in groups of three for this exercise.

(i) Examination of Plant Specimens

Carefully examine plant specimens provided and note symptoms of disease seen.

(ii) Macroscopic Technique

Perform technique used to diagnose the presence of the bacterial wilt pathogen in the vascular tissue of tomato plants provided.

(iii) Biochemical Tests

Each group of students is provided with two (2) unknown bacteria. The purpose of this exercise is to identify the unknown bacteria by performing the biochemical tests and using the key provided, record all information on sheet provided.

1. Record colour of bacterial colonies on agar plates provided.
2. Do the Gram Stain test using the bacteria on agar plates provided. Examine under the microscope (100 x oil immersion) (see page). Record shape of bacteria and whether it is gram-positive, +ve; or gram-negative, -ve.
3. Do Kovac's Oxidase Test. Use indicator solution marked "K" (see page). Record colour change as +ve, positive, -ve, negative: or delayed positive.
4. Examine King's Medium B or KB plates for diffusible pigment. UV lamp is useful for this test (see page).
5. Acid production from Glucose Test. Record colour change of blue to yellow as positive. Presence or absence of gas bubbles in medium (see page).
6. Examine potato slices. Soft feel when prodded indicates positive pectolytic activity.
7. Test for production of nitrite from nitrate. Use indicators marked A and B. Record colour change as positive (see page).
8. Examine Soluble Starch Agar plates (or SSA) for growth and add about 1 ml of iodine solution and record any clear zone around bacterial growth i.e. zones of starch hydrolysis (see page).

The combined results will be tabulated and the key used to identify all the cultures as far as possible.

- If no nitrite is detected, a small amount of zinc dust (not more than 5 mg/ml of culture) is added. A red colouration after a few minutes indicates the presence of nitrite.

  NO_3 + Zinc ⟶ nitrite - NO_2. ∴ does not reduce to nitrite.

- If after the addition of the zinc dust, no colouration is noted this indicates the absence of nitrate which is presumed to have been reduced to nitrite and further reduced. The culture is therefore presumed to be able to reduce nitrate to nitrite. A second culture is incubated for a shorter time to confirm this.

9.7 Soluble Starch Agar Test

Certain plant pathogenic bacteria are able to hydrolyze starch by the use of certain enzymes such as X and B - amylases. Useful test for some *Xanthomonas* spp.

Materials

Nutrient Agar + 0.2% soluble starch, prepared and poured into petri plates. The bacteria are streaked with a wire loop unto the surface and incubated for 3 - 5 days. An uninoculated plate is included as the control.

Procedure

After incubation, the petriplate is flooded with Gram's iodine solution. With a negative reaction, the medium turns blue indicating the presence of starch in the medium. If, however, the bacteria does hydrolyze starch, the blue colouring does not occur around the bacterial colonies.

10. PRACTICAL EXERCISE IN BACTERIOLOGY

Introduction

The purpose of this exercise is to familiarise the student with the basic techniques and methods used in the study of bacteria. This will include the:

- examination of diseased plant material
- macroscopic techniques
- biochemical tests

Since most of the biochemical tests used in the diagnosis of bacteria require at least 48 hours to be completed, many of the tests have been initiated and all that is required is the addition of the reagent and recording the necessary changes that occur.

of each slice and inoculate heavily with bacteria from a 24 hour culture. Include a non-inoculateld slice for each tuber used.

Incubate for 24 hours. The slices are tested for softening with needles. A slight rot only at the point of inoculation is negative.

Useful test for *Erwinia carotovora* group.

9.6 Nitrate Reduction to Nitrite Test

This test is particularly useful for distinguishing *Xanthomonas* spp. which do not reduce nitrate, from many of the yellow saprophytes that do.

Materials

Bacteriological peptone	10 g
Yeast extract	1 g
K_2HPO_4	5 g
KNO_3	1 g
Agar	2 g

Dissolve ingredients and make up to 1 litre with distilled water, and sterilize in 10 ml aliquots in screw-capped bottles at 15 psi for 15 minutes.

Inoculate bottles by stabbing a loopful of bacterial growth through to the base. Incubate at 28°C for 2 days.

Use uninoculated bottles as controls.

Procedure:

The test for nitrate reduction to nitrite is as follows:

(i) A drop of Gram's iodine solution is added to oxidise any hydroxylamine which may be present.

(ii) Solution A - 1 ml of a 0.6% (w/v) solution of dimethyl - x-naphthylamine in 5N Acetic Acid is added.

(iii) Solution B - Followed by 1 ml of a 0.8% (w/v) solution of sulphanilic acid in 5N Acetic Acid.

- If a red colouration develops at the surface in a few minutes, nitrite is present. Therefore Bacterium reduces nitrate to nitrite.

9.4 Acid Production from Glucose Test

The test not only indicates whether the bacterium can utilize glucose as a carbon source but also indicates if the glucose is fermented (anaerobic) or oxidised aerobically.

Materials

Peptone	2.0 g
NaCl	5.0 g
KH_2PO_4	0.3 g
Agar	3.0 g
Bromothymol blue (1% aqueous solution)	3.0 ml
Distilled water	1 litre

The ingredients are dissolved and adjusted to PH 7.1. Add 5 ml. of basal medium to each test tube (or Mc Cartney bottle) and sterilize at 121^{o}C for 20 minutes.

Prepare a 10% aqueous solution of glucose and sterilize by filtration. Add 0.5 ml of the sterile glucose aseptically to each of the tube of basal medium.

Procedure:

Inoculate tubes in duplicate with a long wire stabbed down to the base of the medium with bacteria.

One of each pair of tubes is then sealed with at least 1cm thickness of sterile mineral oil to give anaerobic conditions.

After 3 - 7 days, a colour change from blue to yellow in open tubes indicates that glucose has been oxidised aerobically. If glucose is fermented (anaerobic), the indicator in both tubes changes to yellow.

If the glucose is not attacked, the medium will often turn blue because of the alkali released from the peptone.

Gas production is observed as bubbles trapped in the semi-solid medium.

9.5 Potato Soft Rot Test

Some plant pathogenic bacteria are capable pectolytic activity i.e. able to soften and break down plant tissue. The potato soft rot test is useful in identifying these bacteria.

Cut 7 - 8mm thick slices from washed, alcohol flamed, peeled potato tubers. Place each slice in a Petri dish and add sterile distilled water until about 3 - 4mm deep in the dish. Make a nick in the centre

A visible amount of the fluorescent pigment is readily produced on King's Medium B when viewed under UV light.

Materials - King's Medium B

Proteose Peptone No. 3	20.0 g
$K_2HPO_4 . 3H_2O$	2.5 g
$MgSO_4 . 7H_2O$	6.0 g
Agar	15.0 g
Glycerol	15.0 ml
Distilled water	1 litre

Procedure:

The ingredients are dissolved and made up to one (1) litre with distilled water, adjusted to PH 7.2 with 40% sodium hydroxide solution and sterilize.

Plates are poured, dried and the bacteria spotted or streaked unto the surface and incubated at 28 - 30°C for 3 days. Plates are examined under UV light for pigment formation.

9.3 Kovac's Oxidase Test

The oxidase test of Kovacs may be applied with advantage to most Gram-negative cultures.

Procedure:

As inocula, it is recommended that a 24 hour growth of bacteria on nutrient agar supplemented with 1% glucose be used.

A small visible amount of this bacterial growth is taken with a sterile platinum loop and smeared on to a filter paper that has been dampened with a little freshly made:

1% aqueous tetra-methyl-p-phenylene diamine dihydrochloride. A dark-bluish violet colour formed at the smear within 10 seconds is a positive result.

Definitely negative organisms will not produce the colour even after 5 minutes.

Delayed positive organisms usually give colour after 15 - 60 seconds e.g. many *Xanthomonas* spp.

N.B. A platinum loop is recommended since traces of iron can calalyse the oxidation of the phenylene-diamine compound

Procedure:

The major points in successful staining are as follows:

a. Use reagents less than one year old.
Iodine if not kept in a brown bottle or in the dark will become ineffective.

b. Use freshly grown bacteria.

c. Bacterial smears must be evenly spread out and should not clump together.

Staining Procedure

a. On a clean slide, spread a thin film of bacteria
Dry in air, then lightly flame the underside of the slide twice to fix the bacteria to the slide.

b. Flood the smear with the crystal violet solution for 1 minute.

c. Wash in tap water for a few seconds.
Drain off excess water and lightly blot dry on a paper towel.

d. Flood the smear with iodine solution for 1 minute.

e. Wash in tap water for a few seconds; blot dry.

f. Decolourize with solvent ethyl alcohol, until the solvent flows colourlessly from slide. Blot dry.

g. Rinse slide in tap water for about 2 seconds.

h. Counterstrain for about 10 seconds with safranin solution.

i. Wash in tap water, briefly.
Blot dry and examine under microscope using X100 oil immersion lens.

9.2 Fluorescent Pigment Test

The production of fluorescein, yellowish-green fluorescent pigment which diffuses into the medium, is characteristic of an important group within the genus *Pseudomonas*.

This group contains many important plant pathogens e.g. *P. marginalis* and *P. syringae*.

The production of the fluorescent pigment depends on cultural conditions and is not usually visible on nutrient agar.

The Gram stain is essential for differentiating bacteria into two broad groups:

Gram-positive and Gram-negative. Gram-positive bacteria retain the primary dye, crystal violet, giving a purple to blue-black appearance, while Gram-negative bacteria take up the colour of the counter stain, safranin, and appear red in colour.

Materials Needed

(i) Hucker's Ammonium oxalate crystal violet

Crystal violet dye	2.0 g	Solution A
Ethyl alcohol (95%)	20.0 ml.	" "
Ammonium oxalate	0.8 g	Solution B
Distilled water	80.0 ml	" "

Mix solutions A and B. Store 24 hours before use.

Filter through paper into storage bottle.

(ii) Lugol's Solution - Gram's modification

Iodine	1.0 g
Potassium iodide	2.0 g
Distilled water	300.0 ml

Allow iodine solution to dissolve several hours or overnight in a dark place. Alternately grind the dry iodine and KI in a mortar, adding water slowly until both are in solution. Rinse the remaining solutions with remaining water into a dark bottle.

(iii) Discolourizer

Ethyl alcohol	95%

(iv) Counterstain

Stock solution:

Safranin O	2.5 g
Ethyl alcohol 95%	100.0 ml

Working solution:

Stock solution	10.0 ml
Distilled water	90.0 ml

14.	Acid from glucose aerobically and anaerobically	Not a plant pathogen	
	Acid from glucose aerobically only	*Pseudomonas* spp. (including some plant pathogens)	
15.	Acid from glucose aerobically and anaerobically	*Erwinia* spp.	
	Acid from glucose aerobically only		16
16.	Nitrate not reduced to nitrite		17
17.	Kovacs oxidase negative (delayed positive)	*Xanthomonas* spp.	
18.	Colonies other colours	Discard	

9. LIST OF USEFUL DIAGNOSTIC TESTS FOR IDENTIFYING PLANT PATHOGENIC BACTERIA

9.1 Gram Stain

9.2 Fluorescent Pigment Test

9.3 Kovacs Oxidase Test

9.4 Acid Production from Glucose Test

9.5 Potato Soft Rot Test

9.6 Nitrate Reduction to Nitrite Test

9.7 Soluble Starch Agar Test

9.1 Gram Stain

This test is related to the structural and chemical properties of the cell wall of the bacterium, and serves as a rapid and very basic step in the identification of plant pathogenic bacteria.

The Gram Stain is very important when identifying an unknown bacterium and should not be omitted.

The stains used in this test are not called Gram, but are really a violet dye - Crystal violet and a red dye, Safranin. The test was named after the Danish physician, Gram, who devised it in 1884.

3.	Spores present	*Bacillus* spp.	
	Spores absent		4
4.	Cells more than 0.8u wide	Not a plant pathogen, discard	
	Cells less than 0.8u wide	Possibly *Corynebacterium* spp.	
5.	Cocci, all cells round	Not a plant pathogen, discard	
	Rods, or cocci-bacilli		6
6.	Spores present	*Bacillus* spp.	
	Spores absent		
7.	Colonies white, grey or buff in colour		8
	Colonies yellow		15
	Colonies, other colours		18
8.	Produces fluorescein pigment on Medium B		9
	No fluorescein pigment on Medium B		10
9.	Kovacs oxidase negative	*Pseudomonas* Group I or II.	
	Kovacs oxidase positive	*Pseudomonas* Group III - V	
10.	Kovacs oxidase negative		11
	Kovacs oxidase positive		14
11.	Acid from glucose aerobically and anaerobically		12
	Acid from glucose aerobically only	Some less common plant pathogens maybe keyed out here	
12.	Soft rots potato tissue	*Erwinia carotovora* Group	
	No soft rot of potato tissue		13
13.	Nitrate reduced	Possibly *Erwinia* spp.	
	Nitrate not reduced	*Erwinia amylovora* Group	

symptom development should be used since old decayed tissue usually contains few pathogens and many saprophytes.

With vascular wilts, it is recommended that small pieces of infected stem are used for isolation.

7.2 Preparation of Material

Roots and parts of tissue contaminated with soil should be gently washed with clean water soon after collection. It is essential to surface sterilize the plant tissue to remove all unnecessary surface contaminants. A useful sterilizing agent is a 10% mixture of a commercial chlorine bleach solution. The selected plant material for isolation is left for 2 - 3 minutes in the bleach solution and then thoroughly rinsed in sterile water.

7.3 Preparation of Bacterial Suspensions

A small piece of diseased tissue is removed aseptically from the edge of a typical lesion at the boundary with healthy tissue, or from the vascular system or other suitable place depending on the disease.

This tissue is then surface sterilized and thoroughly rinsed in sterile water.

The material is then aseptically teased apart in about 5 times its own volume of sterile water to allow the bacteria from the tissue to diffuse out into the water. It is advised to leave the material in the water for about 15 - 20 minutes. However, if left too long, there is the possibility of saprophytes contaminating the suspension. Loopfuls of the bacterial suspension are then streaked on to agar plates.

7.4 Medium Used for Growth of Bacteria

Nutrient agar is a general purpose and suitable medium for the isolation of most plant pathogens. This agar is available commercially in a dehydrated form.

The agar plates with the bacterial suspension are then incubated for 48 - 72 hours. Visible colonies of bacteria are seen after this time and can be characterised by its shape, colour and size of colonies.

8. SIMPLIFIED DIAGNOSTIC KEY TO ASSIST IN THE SCREENING OF BACTERIA ISOLATED FROM PLANTS[a]

1.	Gram-positive		2
	Gram-negative		5
2.	Cocci	Not a plant pathogen discard	
	Rods		3

[a] Based on key devised by J.F. Bradbury, 1970.

The absence of non-bacterial pathogens should be confirmed at this point.

6.2 What to look for

Bacteria are slime producing organisms and under moist and cool conditions, their presence can be distinguished as a shiny ooze on the infected plant tissue. This ooze is, however, only visible under cool conditions and is readily seen early in the morning. As soon as the temperature rises the ooze dries up leaving only a film on the surface of the plant tissue.

The presence of bacterial ooze is a useful diagnostic test for those bacterial pathogens inciting leaf spot diseases.

Vascular wilt diseases incited by bacteria, however, do not produce bacterial ooze on the plant tissue but is readily seen when the vascular tissue of the plant is exposed under water.

6.3 Technique Used for Vascular Wilt Bacterial Pathogens

This technique only applies to those bacteria which cause vascular wilt diseases e.g. bacterial wilt of solanaceous crops and bacterial wilt of plantains and bananas.

Example using wilted tomato plant.

The base of the stem/root region of the tomato plant is removed, about 6 cm. long, and this tissue is washed in water to remove soil etc.

This stem tissue is split into two parts exposing the vascular tissue which may or may not show some discolouration.

The 2 pieces of tissue are placed cut side down in a petri plate and flooded with water. The plant is left undisturbed for 20 - 30 minutes after which a visible cloudy bacterial suspension is seen oozing out from the cut surfaces.

It is recommended that the petri dish (or any glass dish) be placed on a black background, so that detection of the cloudy suspension is easily detected.

7. TECHNIQUES USED IN THE ISOLATION OF BACTERIA FROM DISEASED PLANTS

The method normally used for isolating bacteria differs from that used for fungi, and involves the procedure of streaking loopfuls of bacteria from the infected material unto agar plates.

7.1 Choice of Material

In any isolation of bacteria, it is generally better to use recently collected material, still fresh and green. The earliest stages of

Source and Spread	-	Infected planting material. Spread by windblown rain, and also by insects and pruning tools. Bacteria can survive in soil.
Control	-	Use of clean planting material. Crop rotation - at least 1 year. Tolerant varieties. Improved soil fertility. Crop sanitation.

F. Soft Rot of Vegetables

Causal Organism	-	*Erwinia carotovora*
Host Range	-	Most succulent vegetables and root tubers.
Symptoms	-	Generalised soft rotting accompanied by production of slime, and foul odour.
Source and Spread	-	Mainly by water on wounded or senescing tissue. Of importance in the rainy season
Control	-	Crop sanitation.

6. TECHNIQUES AND METHODS USED IN THE DIAGNOSIS OF PLANT PATHOGENIC BACTERIA

The diagnosis (and identification) of plant pathogenic bacteria is a somewhat elusive procedure and quite different from that of fungi. Since most bacteria look alike in form and structure under the microscope, other tests are necessary to identify the organism, most of them not involving the use of a microscope.

These tests are based on the biochemical properties of bacteria and involve the use of media, stains, indicators and reagents. The reactions of the bacteria to these various tests are recorded and with the use of a diagnostic key, the bacterium may be identified.

However, the most important procedure in the diagnosis of disease problems is the careful examination of the diseased specimen.

6.1 Examination of Plant Material

The first step in diagnosis is a thorough examination of the symptoms seen on the diseased plant. This is worth doing as it is often a great aid to future identifications, and the information gained can save work by narrowing down the search to one of a few likely organisms. The specimen should be examined if possible, both in the field and in the laboratory by the unaided eye and by handlens or dissecting microscope.

Control	-	Use of disease free suckers. Eradication of infected stools. Sterilizing of cutting tools. Removal of *Heliconia* spp. from cultivation site.
		Removal of male flower bud. Crop rotation.

D. Black Rot Disease of Crucifers

Causal Organism	-	*Xanthomonas campestris* pv. *campestris*
Host Range	-	Crucifers: especially cabbage.
Symptoms	-	Damping-off of seedlings in nursery.
		Marginal V-shaped lesions associated with chlorotic leaf tissue and blackening of veins. Malformed, black and decayed heads. N.B. Vary according to degree of tolerance/susceptibility of variety.
Source and Spread	-	Both seedborne and soilborne. Movement of infected seedlings to planting site.
		Rain splash and irrigation water. Ploughing in of infected crop residues in soil.
Control	-	Use of clean seed.
		Crop rotation - 1½ - 2 years. Tolerant varieties.
		Removal of all diseased material from field.

E. Cassava Bacterial Blight of Cassava

Causal Organism	-	*Xanthomonas manihotis*
Host Range	-	Cassava
Symptoms	-	Leaf spotting with angular water soaked spots brown or reddish-brown in colour. Spots coalesce resulting in blighting of leaf. Gum exudation and cankers on stem, wilting of leaves and death of shoot

Symptoms	-	A general wilt of the entire plant, usually with collapse of the young stem tissue; healthy root system. Sections of lower stem may show vascular discolouration. Diagnostic bacterial streaming from cut vascular tissue
Source and Spread	-	Soil inhabiting pathogen; spread by movement of infected plant and soil
Control	-	Crop rotation and sanitary practices. Use of pulverised limestone as a soil amendment. Tolerant varieties.

C. Bacterial Wilt of Plantains and Bananas

Causal Organism	-	*Pseudomonas solanacearum* Race 2.
Host Range	-	Plantains, Bananas and *Heliconia* spp.
Symptoms	-	(i) Soilborne Strain: wilting of plant beginning with premature yellowing of leaves, blackening and dieback of suckers; discolouration (mostly reddening) of veins in corm and pseudostem (ii) Insectborne Strain: blackening and dieback of male buds, uneven ripening and premature yellowing of fruit, occasional blackening of fingers, internal dry rot of fruit tissue
Source and Spread	-	(i) Soilborne Strain: soil inhabiting pathogen: infected suckers, root to root transmission, bacterial smears on cultivation tools. (ii) Insectborne Strain: spread by flower visiting insects, infected suckers etc.

(iv) Crop Sanitation Practices:

This involves the careful removal of infected plant material so as to reduce the incidence and spread of the disease. These include for example:

- removing infected plants from the field
- preventing infected crops residues being ploughed into the soil
- sterilizing of cutting and pruning implements

(v) Tolerant or Resistant Varieties:

The use of varieties with known and tested tolerance to the particular disease is perhaps one the few control measures which can benefit the farmer in the short term. The use of blackrot tolerant varieties of cabbage has shown promise in reducing losses especially during the rainy season production.

5. SOME EXAMPLES OF BACTERIAL DISEASES OF FOOD CROPS

A. Peppery Leaf Spot Disease of Crucifers

Causal Organism	-	*Pseudomonas syringae* pv. *maculicola*
Host Range	-	Crucifers especially cauliflower and cabbage
Symptoms	-	Strictly a leaf spotting pathogen. Causes small brown water-soaked tiny spots on underside of lower leaves. Spots may induce malformation of leaf; spots coalesce and result in defoliation
Source and Spread	-	Seedborne: can survive in soil. Infection occurs through stomata. Bacterial exudates are readily spread by water and wind.
Control	-	Use of clean seed, Crop Rotation. Tolerant varieties

B. Bacterial Wilt of Solanaceous Crops

Causal Organism	-	*Pseudomonas solanacearum* Race 1.
Host Range	-	All solanaceous crops - tomato, sweet and hot peppers and melongene

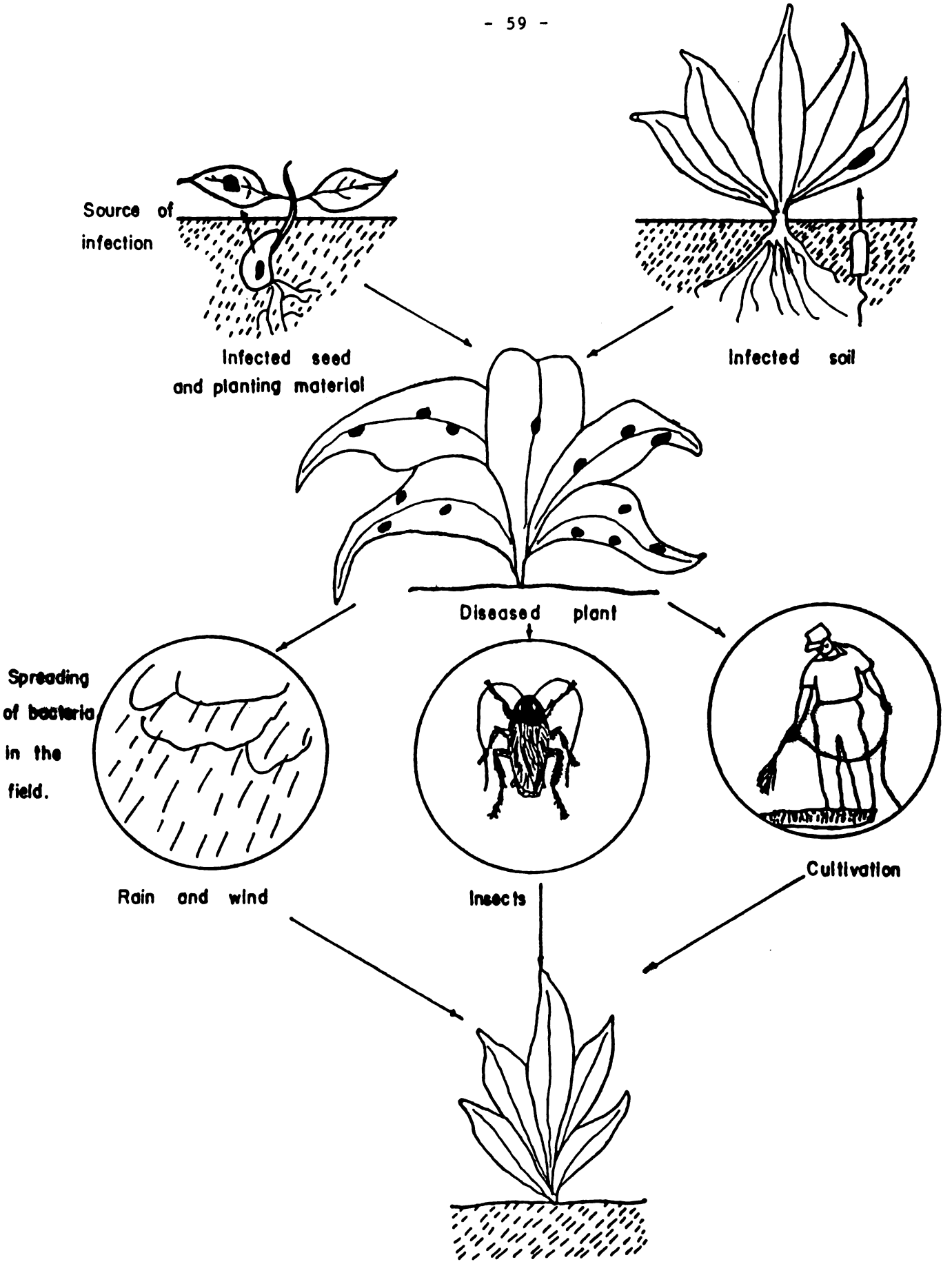

Fig 4 Source of infection and spreading of bacteria in the field

Adapted from: Methods in Plant Pathology by Z. Kiraly, Z. Klement, F. Solymosy and I. Vörös

The disease developed from the seedling or the young plant infected by the soil, may spread to the neighbouring plants. Generally the spread of the disease begins from such small centres of infection and the disease spreads over the whole field under favourable conditions (Fig. 4).

If the disease centre is present, further rapid spread of the disease is aided by environmental conditions, thus rainy, humid and warm weather has an important role in the epidemic spread of bacterial diseases. Of special importance is the spread of bacterial diseases by wind blown rain.

Spread of bacterial diseases can also occur by:

(i) Insects visiting the infected tissue and picking up bacteria in its mouth parts e.g. insectborne strain of *Pseudomonas solanacearum*.

(ii) Cultivation practices - e.g. removal of trash leaves can carry bacterial smears on the cutting implement and be transmitted to healthy plants e.g. Bacterial Wilt of plantains and bananas.

4. CONTROL OF BACTERIAL DISEASES

In controlling bacterial plant pathogens, the same difficulties are faced as in the control of viral diseases. The bacteria multiplying inside the plant tissue cannot be reached effectively by chemicals or antibacterial compounds. Therefore control of bacterial diseases is primarily based on preventive measures to keep the pathogen away from the plant.

The control of bacterial plant pathogens may be summarized as follows:

(i) Use of Clean Seed:

This is one of the most important means of controlling bacterial seedborne diseases. In cases where the percentage infection of seed is low, use of a hot water treatment may be recommended e.g. in control of blackrot disease of cabbage.

(ii) Use of Disease-Free Planting Material:

For those crops propagated vegetatively, selection of disease-free material is of extreme importance, since diseases can spread to new locations by the use of infected vegetative material. e.g. cassava bacterial blight of cassava.

(iii) Crop Rotation Practices

Continuous cultivation in a particular area may lead to the establishment of the bacterial pathogen in the soil. In situations where this has occurred, crop rotation systems should be introduced over an average period of 1 - 2 years so as to reduce the levels of the plant pathogenic bacteria in the soil. e.g. blackrot of cabbage and bacterial wilt of solanaceous crops.

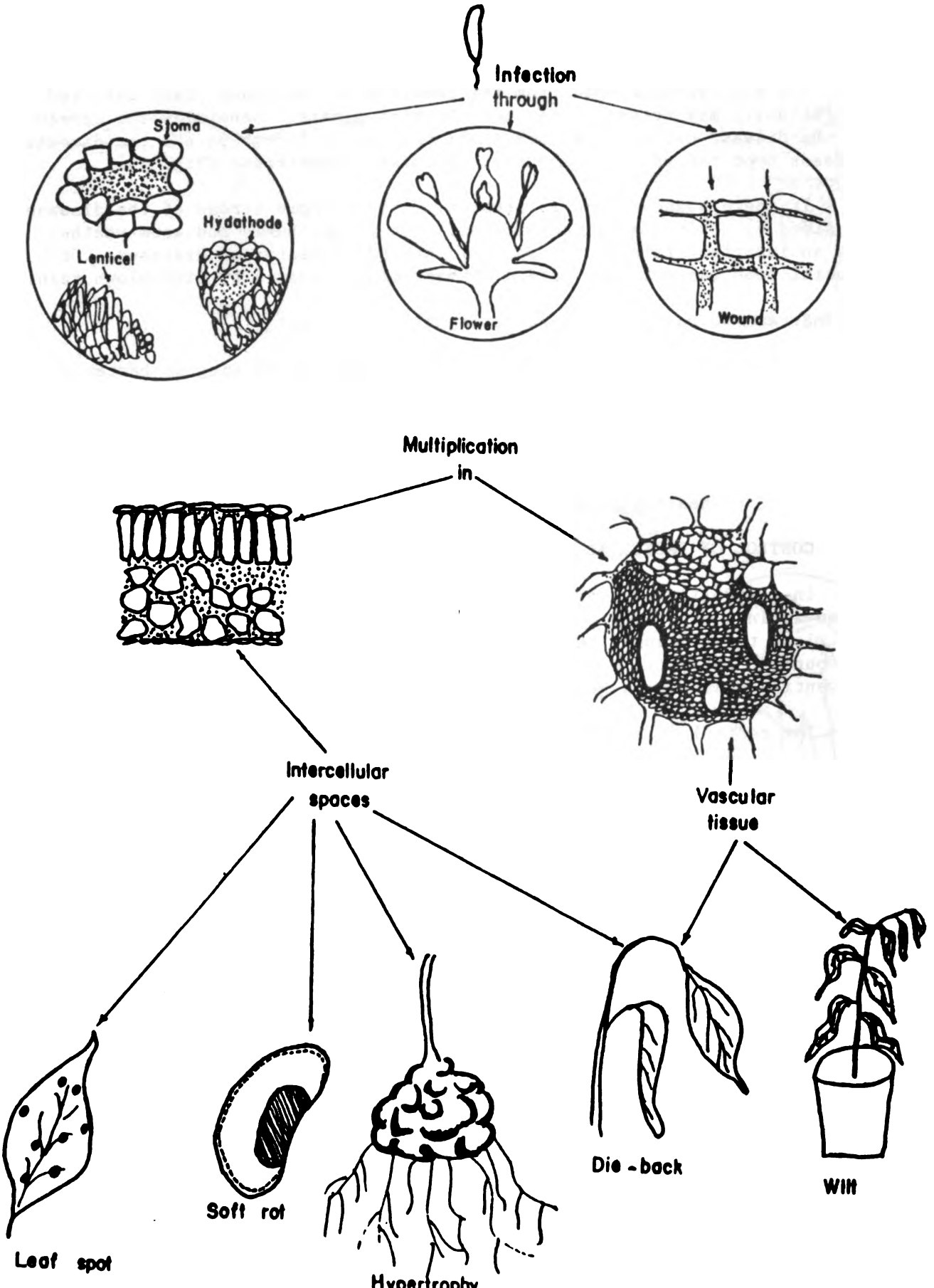

Fig. 3 Modes and places of infection, multiplication of plant pathogenic bacteria and types of symptoms

Adapted from: Methods in Plant Pathology by Z. Kiraly, Z. Klement, F. Solymosy and J. Voros

2.2 Vascular Wilts

Wilting of the plant occurs when bacteria have entered and attacked the vascular tissue, blocking the transportation of both nutrients and water. The bacteria enter the plant via wounds in the root system and move directly into the vascular tissue where they multiply and rapidly spread (Fig. 3). Discolouration of the vascular tissue is sometimes visible e.g. bacterial wilt of solanaceous crops, *Pseudomonas solanacearum*.

2.3 Vascular and Parenchymatous - type symptoms

This group involves those bacterial diseases which are characterised by various symptoms ranging from leaf spots, vascular infection and dieback. Infection usually takes place via leaf tissue and the disease produces distinct leaf spot symptoms. However, the pathogen then enters the vascular system and progresses to produce a systemic infection leading to wilts, dieback and death of the entire plant e.g. cassava bacterial blight, *Xanthomonas manihotis*.

2.4 Soft Rots

These pathogenic bacteria causing soft rots of succulent plant tissue produce pectin-decomposing enzymes capable of breaking down pectin holding plant cells together. This leads to the plant tissue becoming disorganised and soft. The rot is usually completed by secondary fungal and bacterial colonizers e.g. soft rots of onions, carrots and crucifers, *Erwinia cartovora*.

3. SOURCE AND SPREAD OF THE PATHOGEN IN THE FIELD

The appearance of the bacterial disease in the field has two sources, the infected plant material and/or the infested soil (see Fig. 4).

The bacterial pathogen enters the field with infected seed, infected seedlings or shoots. In the case of the seed being infected, the pathogen shelters itself either by adhering to the seed coat, or under the seed coat e.g. *X. campestris* pv. *campestris* on cabbage seed.

In diseases of woody plants or shrubs, the bacteria usually remain in infected stems, from where they begin to multiply when the infected stems are planted e.g. *X. manihotis* of cassava.

The disease in the field can also originate from the soil which has been contaminated with the pathogenic bacteria over the years. Numerous plant pathogenic bacteria are able to remain in the soil for long periods of time. These bacteria reach the soil by means of infected plant material falling on the soil or by the incorporation of infected material in the soil. The observance of crop rotation has an important role in the case of infested soils.

added to watery suspensions of the incitant bacteria, produces various effects e.g. agglutination, precipitation or the bacteria may be destroyed.

This technique is used for differentiating species and strains of bacteria which cannot otherwise be distinguished by ordinary laboratory methods.

1.8 Plant Pathogenic Bacteria - Genera

Plant pathogenic bacteria are found within a few genera e.g.

Pseudomonas

Xanthomonas

Erwinia

Corynebacterium : not common in the tropics

Agrobacterium " " " " "

However, within each genus, there are perhaps between 8 - 10 distinct species of pathogenic bacteria inciting diseases within plants.

2. TYPES OF SYMPTOMS CAUSED BY PLANT PATHOGENIC BACTERIA

The symptoms caused by bacteria are often similar to those incited by infections of fungi or sometimes of viruses.

A general characteristic symptom of diseases incited by bacteria, in contrast to viral and fungal diseases, it that the attacked tissues are water-soaked, and when observed in transmitted light, are greasy in appearance; quite often bacterial exudate occurs on the diseased tissue.

The following types of symptoms of bacterial diseases have been grouped together, and can be differentiated as:

2.1 Leaf Spots

Leaf spot diseases generally refer to the situation when death or necrosis of the leaf tissue is localized. In the early stages of bacterial infection, the spots are of a darker green colour than the uninvaded tissue. These spots then appear water-soaked or greasy and may be surrounded by a pale yellow zone originating from the bacterial toxins diffusing into the tissue.

In some cases, infected spots on the leaf fall out, so that the leaves become full of holes, e.g. peppery leaf spot of crucifers, *Pseudomonas syringae*. These individual leaf spot infections usually enlarge and coalesce and the entire leaf is killed.

are attached to the outside of the bags: Location of field; farmer's name; crop; date; plant part or soil. Other information pertinent to the samples taken may be recorded elsewhere. The bags with the samples are placed in an insulated styrofoam box (ice-box) and if the laboratory is located far away from the field then ice is included in the box. If samples cannot be processed in the laboratory immediately then they may be kept in a refrigerator at 5°C for up to one (1) week.

5. NEMATODE EXTRACTION AND STAINING TECHNIQUES

5.1 Extraction of Nematodes from Soil

Several methods are described for extracting nematodes from soil. Two of these methods are described below.

5.1.1 Direct Extraction using Modified Baermann's Funnel Technique

Apparatus: One pair 22cm - diameter pie-pan (one with its base removed and replaced by coarse wire or nylon mesh); double-ply unscented facial handkerchiefs.

a. Remove debris and pebbles from the soil sample and break clods with the fingers. Mix soil thoroughly and take a 100ml sub-sample which is placed in a beaker with water.

b. Two facial handkerchiefs are placed on the mesh of the pie-pan. The 100ml soil suspension is poured onto an inverted watch glass placed at the centre of the facial handkerchiefs resting on the mesh of the pie-pan. The water is allowed to drain and the corners of the facial handkerchiefs are turned in to avoid loss of water.

c. Place the pie-pan containing the soil in pie-pan containing about 200ml water. Add water if necessary to just about flood the soil.

d. Leave standing for 24 - 48 hours, thereafter remove the upper pie-pan and collect the nematode suspension in a 250ml beaker.

5.1.2 Cobb's Gravity and Sieving and Modified Baermann's Funnel Techniques

Apparatus: Series of sieves: coarse mesh, 100 -, 250-, 325 - mesh per sq. in. sieves; two buckets; 250ml beaker; pie-pans; facial handkerchiefs.

a. Place the 100cc sub-sample (prepared as in 5.1.1 (a) into bucket #1 and pour in about six (6) litres of water. Break clods with fingers.

b. Stir and leave for 1 minute. Pour contents through a coarse sieve into bucket #2. Wash residue with squirt of water and collect this water in bucket #2. Discard residues from sieve and bucket #1.

c. Stir contents of bucket #2 and leave for 1 minute. Pour contents through 100 - mesh sieve and collect water in bucket #1. Backwash residue from sieve into a 250ml beaker.

d. Repeat step c with 250 - and 325 - mesh sieves, each time backwashing residues into the same 250ml beaker.

e. Pour suspension onto facial handkerchiefs resting on pie-pan (5.1.1 b-d).

5.2 Extraction of Nematodes from Root

5.2.1 Maceration of roots

Apparatus: blender; scalpel or razorblade; pie-pans; double-ply facial handkerchiefs.

a. Gently wash soil from feeder roots

b. Cut roots into 1 - cm pieces

c. Blend 5 - 10g root pieces in 100ml water for 10 - 20 seconds depending on root texture

d. Proceed as for 5.1.1 b-d (the blended root suspension replaces the soil suspension)

5.2.2 Maceration of roots, tubers, bulbs etc for quick diagnosis

Simply macerate, with the use of dissecting needles, infected tissue in water and examine for nematodes under the dissecting microscope.

5.3 Staining Roots

Materials: Acid-fuchsin in lactophenol (mix 100ml lactophenol and 5cc of a solution made by dissolving 1g acid fuchsin in 100ml water) (cotton blue or aniline blue may be used instead of acid fushsin).

a. Tie 5 - 10g of washed 1cm piece roots, obtained as for 5.2.1 a-b, in a piece of muslin or cheesecloth

b. Dip in a boiling solution of acid fuchsin in lactophenol for 1 minute

c. Wash excess stain in gently running tap water.

d. Place stained roots in clear lactophenol and leave overnight after which the roots are ready for microscopic examination.

6. PROCESSING NEMATODE SAMPLES

6.1 Concentrating Nematode Suspension

The suspension of nematodes collected from the pie-pan should be brought to about 10ml. This is done as follows:

a. The suspension in a 250ml beaker is allowed to stand undisturbed for one hour after which a little more than half is carefully decanted.

b. The suspension is poured into a 100ml beaker, left for 45 minutes after which half is decanted.

c. The suspension is now poured into a 50ml beaker, allowed to stand for 30 minutes after which the top portion is decanted leaving about 10ml of the suspension.

6.2 Killing Nematodes

Viewing live nematodes under the dissecting microscope can be fascinating and characteristics of certain genera can be observed in this exercise. However, for viewing under the light compound microscope and for counting purposes, it is necessary to 'kill' the nematodes. This can be done by:

a. Pouring the nematode suspension into a test-tube which is placed in a water bath at $65^{o}C$ for three minutes, or,

b. Adding an equal volume of boiling water to the nematode suspension

6.3 Fixing Nematodes

When nematodes are 'fixed' they retain most of their features for a long time. Fixatives, therefore, allow for the storage of nematode samples until a convenient time when counting can be done.

Two of the methods recommended for fixing nematodes are:

a. Use of TAF (Triethanolamine formalin). TAF at double strength is made by mixing 4ml triethanolamine, 82ml distilled water and 14ml commercial formaldehyde solution. Ten ml TAF is added to 10ml of the killed nematode suspension (TAF is added to the killed nematode suspension at a 1.1 ratio).

b. Prepare a formaldehyde solution by adding 14ml commercial formaldehyde (38%) to 86ml water. Add 10ml of this to 10ml of the killed nematode suspension (1.1 ratio).

7. IDENTIFICATION OF NEMATODES

After processing, the nematode samples are ready for microscopic examination.

7.1 Examination under the Dissecting Microscope

Before nematodes can be counted, it is necessary to identify the types of nematodes present in the sample. About 1ml of the suspension is taken up with a dropper and released into a syracuse watch glass. After allowing the nematodes to settle, they are examined under the dissecting microscope. A suitable dissecting microscope would allow you to recognise: male and female nematodes; size (relative) and shape of the nematodes; presence of a stylet.

7.2 Temporary Slide Preparation of Nematodes

For identification of the plant-parasitic nematodes even to the generic level, it is important to view them under the light compound microscope. This means that slides have to be prepared of the various nematode types present in the suspension. Nematodes are 'picked' and mounted in water or lactophenol. (If the nematodes are mounted in lactophenol they may become distorted but most of them will recover after some time). Small pieces of glass rod or beads are placed at the periphery of the mounting medium. A cover slip is then applied and the slide may be sealed with nail polish (Fig. 1).

7.3 Picking Nematodes

Reference was made to "picking" nematodes in the last paragraph. Picks can be made by trimming slender bamboo rods to a fine strip at one end or by stripping the vane off the rachis of a quill feather. To pick a nematode, place one hand on the coarse adjuster of the microscope and bring the nematode to the surface with the aid of the pick. Keep the nematode in focus at all time. A final flick of the pick is necessary to break the surface tension of the liquid. The nematode is transferred to the mounting medium on the slide by placing the tip of the pick in the medium.

7.4 Examination under the Light Compound Microscope

There is need for specialized training in the identification of plant-parasitic nematodes. An attempt will be made here to assist in the identification of broad categories of nematodes.

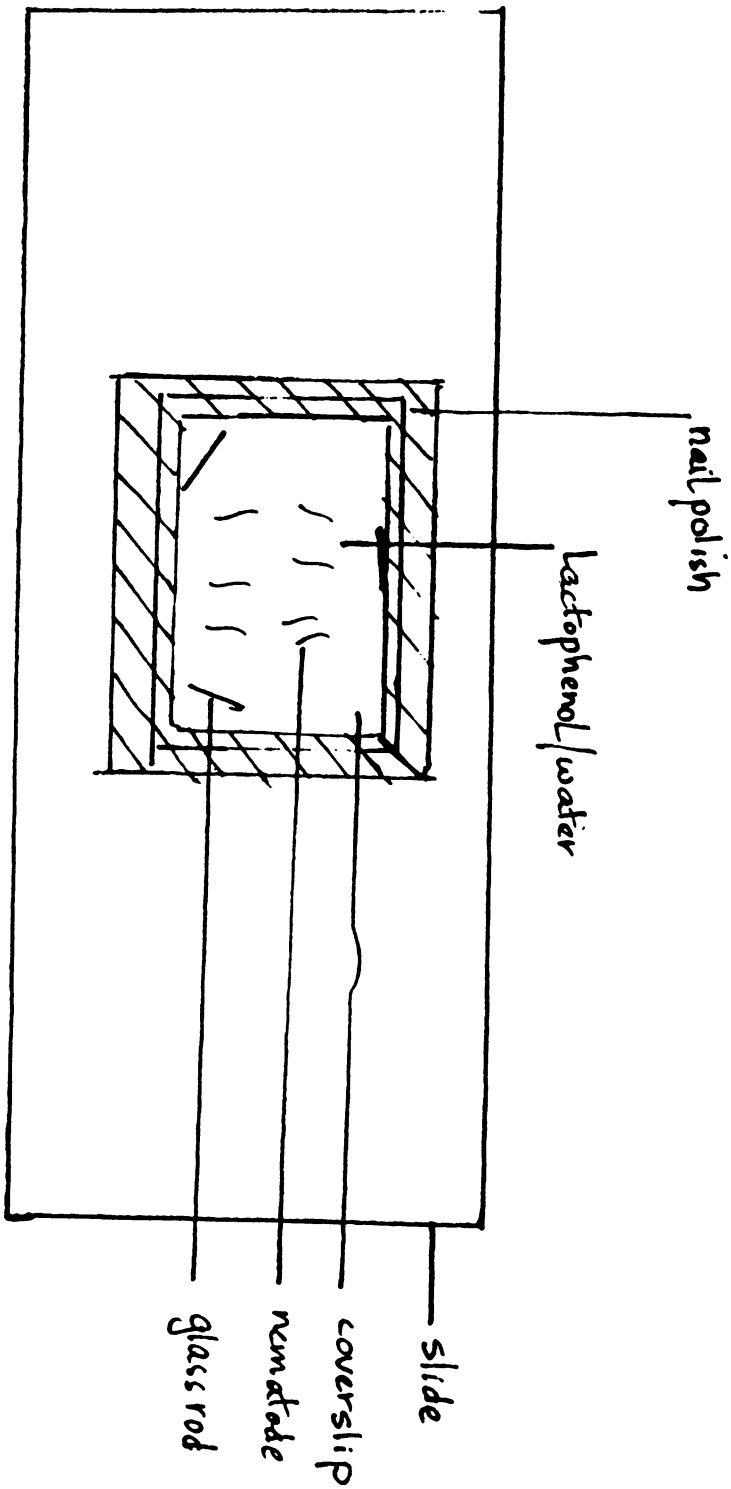

FIG 1. Temporary slide preparation.

Nematodes may be categorised into two basic groups - those which possess no stylets (the free-living nematodes) (Fig. 2) and those which possess a stylet (Fig. 3). With respect to the latter group, it is important to recognise the type of stylet present. The majority of plant-parasitic nematodes possess a stylet with well-developed basal knobs but some nematodes possess stylets with either no basal knobs (Fig. 4) or weakly developed knobs. The fungal feeders fall into the last category. Some fungal feeders e.g. *Dorylaimus* sp. possess two chisel-shaped stylets (Fig. 5).

Of the plant-parasitic nematodes possessing stylets with well-developed knobs, there are two basic groups: those with a two-part anterior intestine, the dorylaimoides and those with a three-part anterior intestine, the tylenchids (Fig. 6 A and B).

The main features of plant-parasitic nematodes are shown in Figure 7. For basic identification of plant-parasitic nematode genera the following features are important: relaxed shape of nematode; size of nematode; presence of males; type of median bulb; length and development of stylet; body outline; position of vulva and number of gonads present; length and shape of tail; relationship of oesophageal glands and intestine i.e. whether there is an overlap and if so, whether the overlap is dorsal or ventral.

8. COUNTING NEMATODES

8.1 Soil, Root and Rhizome Nematode Populations

The nematode suspensions obtained after extraction and processing should be examined for qualitative and quantitative assessment. The latter is done by bringing the suspension to about 200ml by the addition of water. Air bubbles are introduced by means of a small aquarium pump and 3, 5 - 10ml aliquots are taken with a pipette and delivered into counting dishes. The nematodes are allowed to settle for about five minutes and then counted under the dissecting microscope. The mean number of nematodes of the 3 counts is calculated. Plant-parasitic nematode populations may be expressed per litre soil and per 100g root/rhizome. The following is an example of the calculation done in the determination of the nematode population in one litre soil:

Quantity of soil for extraction: 100ml

Therefore multiplication factor for 1 litre

$$\text{Soil: } \frac{1000}{100} = 10$$

Mean no. of nematodes in 5ml aliquots = a

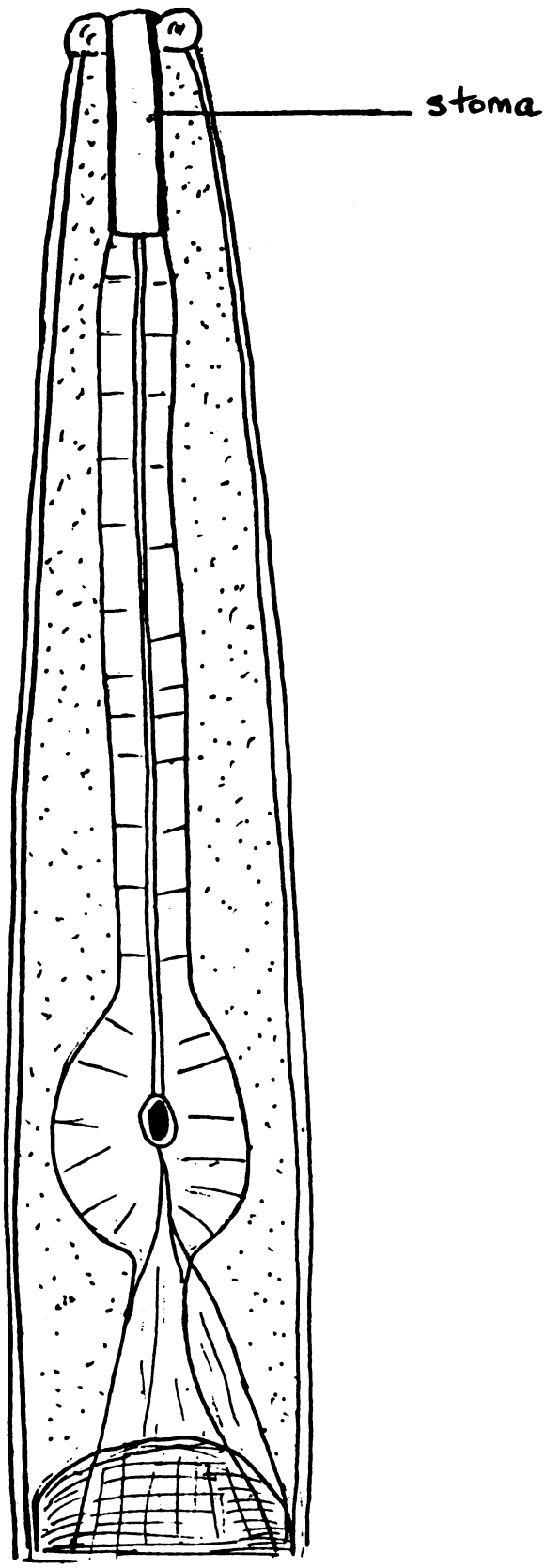

FIG. 2 DIAGRAM OF ANTERIOR

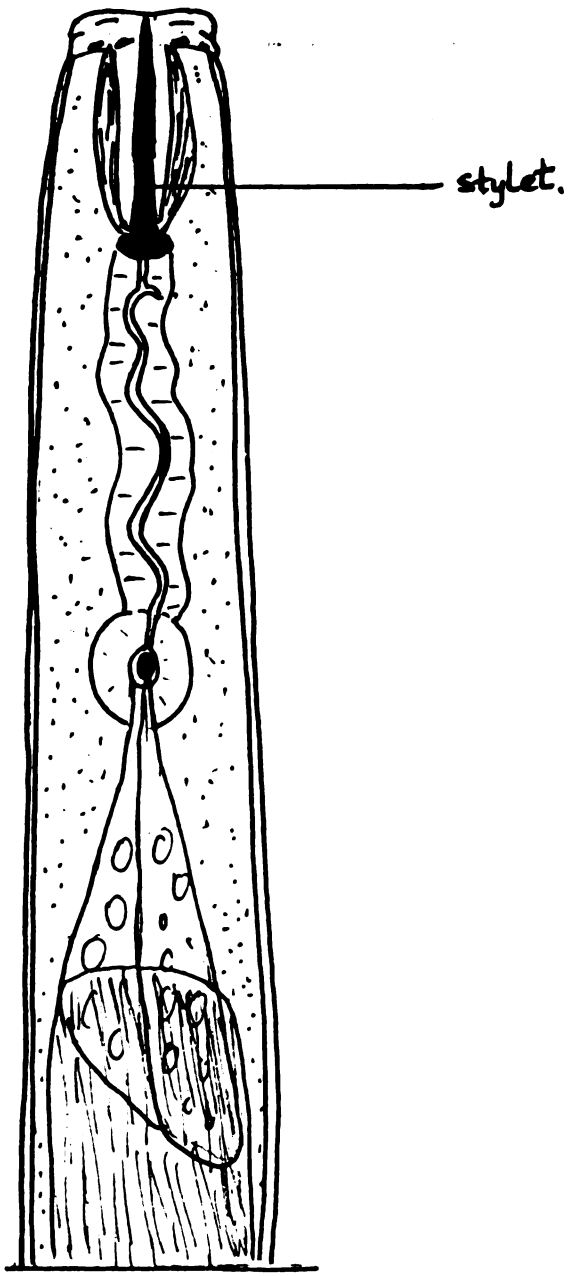

FIG.3 PLANT-PARASITIC NEMATODE (ANTERIOR PART) SHOWING STYLET.

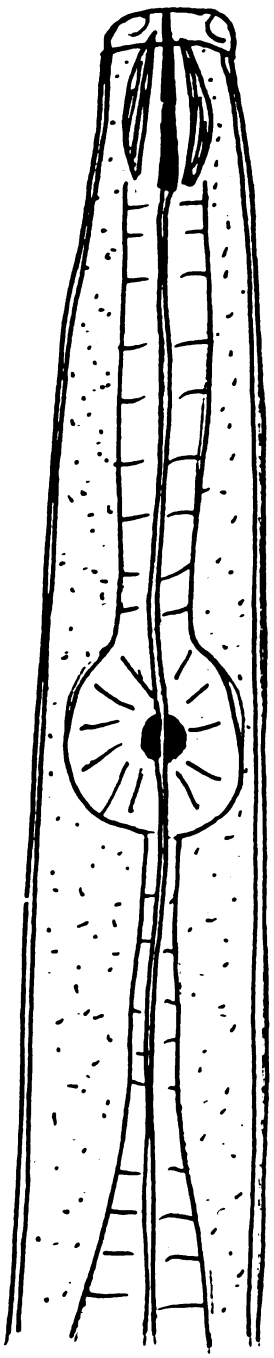

FIG. 4 NEMATODE SHOWING STYLET WITHOUT KNOBS.

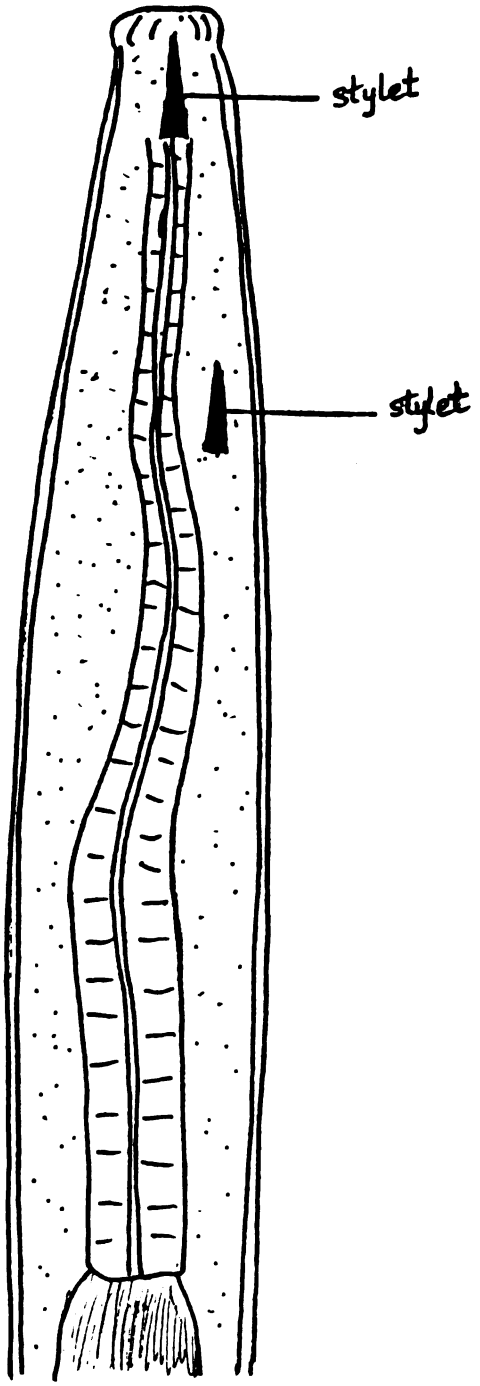

FIG. 5 ANTERIOR PART OF DORYLAIMUS SP.

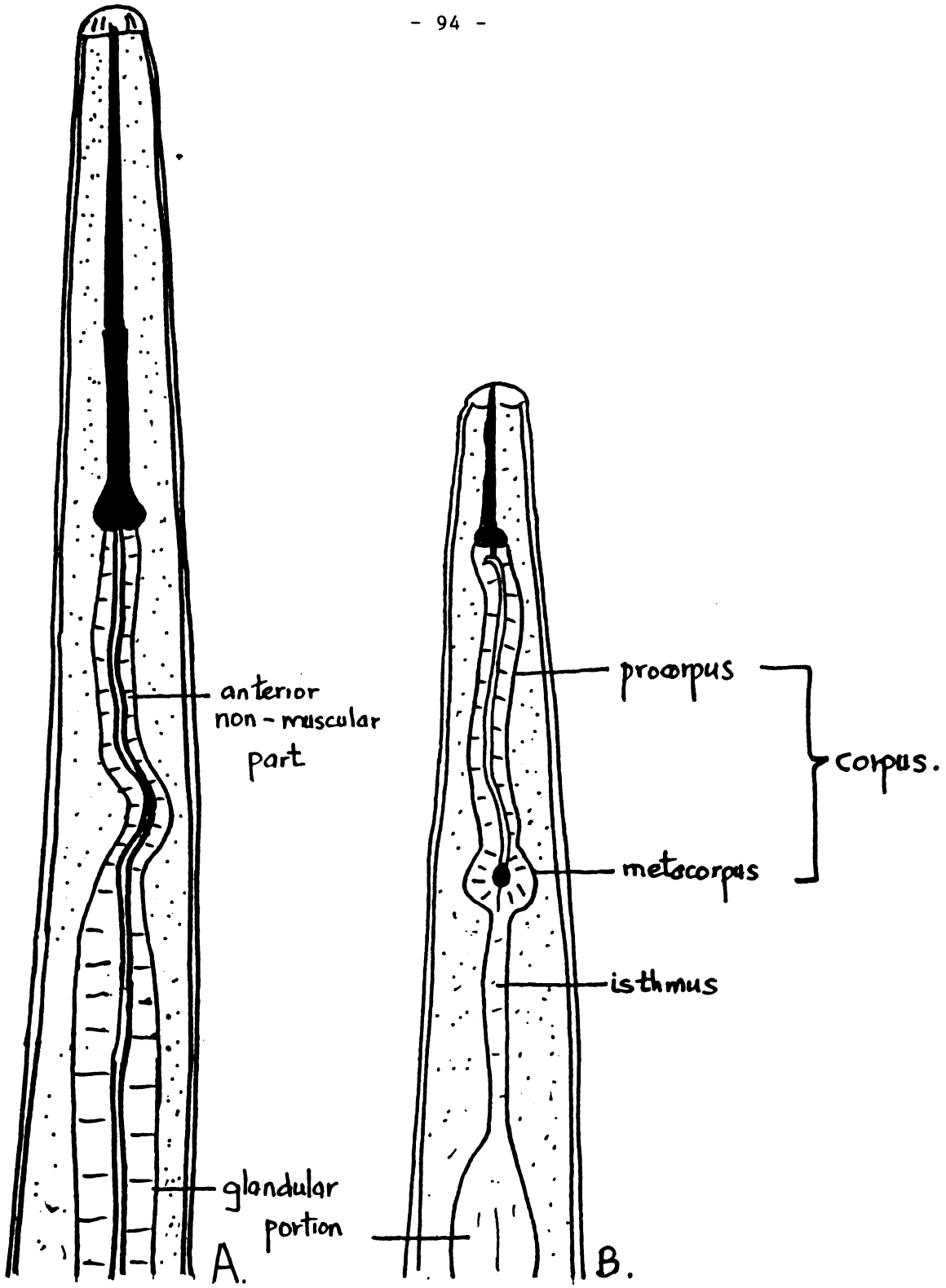

FIG. 6 (A) Nematode with two-part oesophagus
(B) Nematode with three-part oesophagus

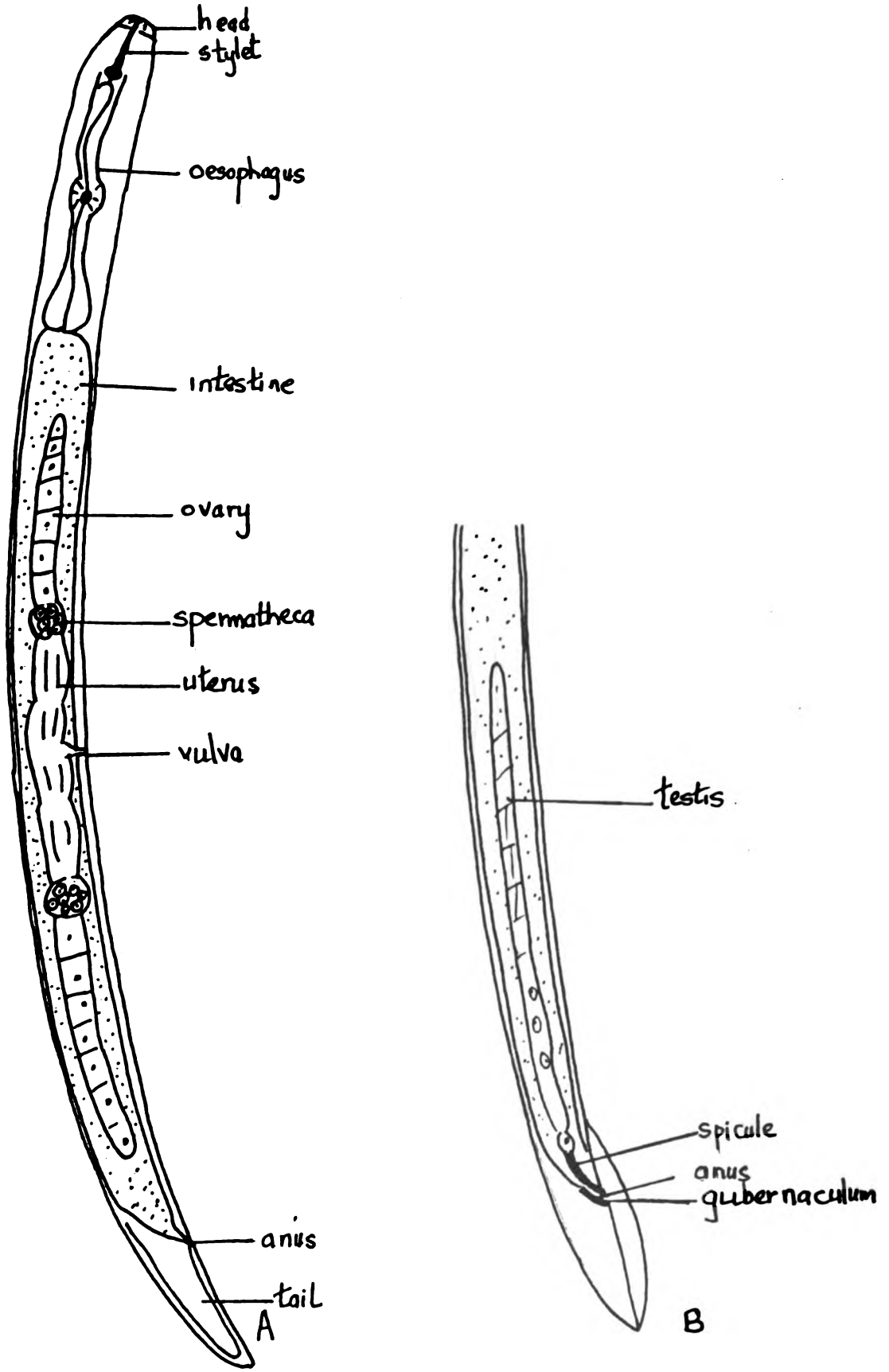

FIG 1. Diagrams of typical plant-parasitic nematodes

A. Female B. Male

Therefore mean no. of nematodes in 200ml = $\frac{200}{5}$ x a

= 40 x a

Population, therefore, per litre soil = 40 x 10 x a

= 400 x a

8.2 Stained Root Nematode Populations

Some nematodes are sedentary but do not induce abnormal growth symptoms of the root system e.g. reniform nematode. Their numbers per quantity of root can be determined by conducting counts under the dissecting microscope.

8.3 Rootknot Nematode Indexing

In cases of galling caused by rootknot nematodes, indexing can be done by simply examining the root system. Scales of 0 - 5 or 0 - 10 have been developed by various researchers. This indexing can be done in the field.

9. NEMATODE - PATHOGEN INTERACTIONS

Many instances have been cited where plant-parasitic nematodes interact with plant pathogens to cause disease. Examples have been reported of plant-parasitic nematodes interacting with viruses, bacteria and fungi resulting in problems more severe than when the organisms occur alone. Rootknot nematodes have been known to interact with *Fusarium* sp., the relationship resulting in a breakdown of *Fusarium* - wilt resistance in the host. It has been shown that the severity of Panama disease of banana caused by *Fusarium oxysporum f.* sp. *cubense* is greater when the burrowing nematode, *Radopholus similis*, is present.

Plant-parasitic nematodes have been shown to transmit bacteria and viruses. The dagger nematodes *Xiphinema* spp., have been shown to be vectors of several important plant viruses. Their role as vectors of viruses in the Caribbean has been given little or no attention.

10. THE DIAGNOSIS AND GUIDELINES FOR RECOMMENDATIONS FOR CONTROL

The diagnosis will be finalised on the basis of:

a. Symptomns observed

b. Nematode populations, both qualitative and quantitative

c. Reference to work done on losses caused by nematodes involving same host under similar environmental conditions

d. Whether plant-parasitic nematodes are primary or secondary agents

e. Whether interrelationship involving nematode - fungi/bacteria, exists

11. STEPS IN DIAGNOSIS OF TWO SELECTED EXAMPLES OF NEMATODE PROBLEMS

11.1 Rootknot, Reniform and other Nematodes associated with tomato

a. Field observation of above-ground symptoms and distribution of affected plants

b. Uprooting of plants and examination of rootsystem for symptoms

c. Rootknot indexing on scale of 0 - 5 or 0 - 10

d. Collection of soil and root samples

e. Extraction of nematodes from soil and root and staining of roots

f. Determination of nematode population densities

g. Comparison of population densities from 'thrifty' and 'unthrifty' plants, where applicable

h. Reference to work published on subject area

i. Final diagnosis and recommendations

11.2 Nematode Problems of Plantains and Bananas

a. Field observations of above-ground symptoms and distribution of affected plants

b. Examination of feeder-roots and collection of feeder-roots and rhizosphere soil samples

c. Uprooting plant and noting symptoms associated with primary and secondary roots and rhizome

d. Collection of samples of primary and secondary roots and rhizomes (where necrosis, if any, is observed)

e. Maceration of rhizomes for quick examination

f. Extraction of nematodes from feeder roots, primary and secondary roots and rhizomes

g. Staining of feeder roots

h. Determination of nematode population densities

i. Comparison of population densities associated with 'thrifty' and 'unthrifty' plants (where applicable)

j. Reference to work published in subject area

k. Final diagnosis and recommendations

12. NEMATODE PROBLEMS OF FOOD CROPS AND THEIR CONTROL

12.1 Nematode Problems

The nematode problems of food crops in the region vary from place to place. The occurrence of nematode problems depends, among other factors, on cropping history and the qualitative and quantitative plant-parasitic nematode populations present. There is a lack of information on the importance of certain types of nematodes e.g. dagger, stunt and lance nematodes, in crop production. With respect to the dagger and lance nematodes, it may be that there is a need to utilise improved selected techniques in the determination of their population densities.

A summary of some of the major nematode problems of food crops is given below.

12.1.1 Rootknot Nematodes

Meloidogyne spp.; *M. incognita; M. javanica; M. exigua;* (the most common being *M. incognita*)

Distribution	-	Throughout the region.
Hosts	-	Vegetables including beans, carrot, cucurbits, solanaceae, lettuce, okra.
Mode of Life	-	Sedentary endoparasitic.
Symptoms	-	Galled or knotted root system; destruction of feeder roots; root rot; wilt, particularly in dry season; stunting; chlorosis; reduced yields.
Importance	-	May be considered to be the most important nematode problem of vegetable crops. Can cause severe yield losses and may even cause death of individual plants or patches of plants.

12.1.2 Reniform Nematode

Rotylenchulus reniformis

Distribution - Throughout the region

Hosts - Vegetable crops including tomato, cucumber and pumpkins, peanuts, soybean and pigeon peas, sweet potato, plantains and bananas

Mode of Life - Sedentary semi-endoparasitic

Symptoms - Root lesions and root rot, stunting, reduced yields

Importance - On its own, this nematode can cause reduced yields, however, in mixed populations its numbers may be depressed by the presence of other nematodes e.g. rootknot nematodes

12.1.3 Ectoparasitic Nematodes

Spiral nematodes - *Helicotylenchus* spp. chiefly
- *H. dihystera, H. pseudorobustus*
- *H. multicinctus Peltamigratus* spp.

Lance nematodes - *Hoplolaimus* spp.

Stunt nematodes - *Tylenchorhynchus* spp.

Ring nematodes - *Macroposthonia* spp.
- *Hemicriconemoides* spp.

Distribution - Variably distributed in the region

Hosts - Wide host range including vegetables, plantains and bananas

Mode of Life - Ectoparasitic

Symptoms - Lesions; damaged roots; root rot; stunting; reduced yield

Importance - Members of this group of nematodes are of varying importance. Spiral nematodes are the most commonly found and can cause some damage on their own. However, most of these nematodes are found in mixed populations and contribute to crop losses.

12.1.4 Dorylaimoides

Dagger nematodes (*Xiphinema* spp.)

Stubby root nematodes (*Trichodorus* spp.)

Distribution - Throughout the region

Hosts - Wide host range including vegetable crops

Mode of Life - Ectoparasitic

Importance - The dagger nematodes are widely distributed and may be of more importance than are appreciated currently. The nematodes can cause serious reduction in growth and yield of crops

12.1.5 The Burrowing and Lesion Nematodes

The burrowing nematode (*Radopholus similis*)

The lesion nematode (*Pratylenchus* spp. including *P. coffeae*)

Distribution - Confined to certain parts of the the Caribbean

Hosts - Mainly plantains and bananas; the lesion nematode has wide host range

Mode of Life - Migratory endoparasitic

Symptoms - Root lesions and necrosis; root rot; rhizome necrosis; toppling; chlorosis; stunting; reduced yield

Importance - The nematodes, chiefly the burrowing nematode, are of great economic importance in the Caribbean. They cause considerable reduction in growth, yield and production cycle of the crop

12.1.6 The Yam and Lesion Nematodes

Scutellonema bradys (the yam nematode)

Pratylenchus coffeae (the lesion nematode)

Distribution - Restricted to some parts of the region

Host - Yam; the lesion nematode has other hosts

Mode of Life - Migratory endoparasitic

Symptoms - Necrosis of tuber

Importance - These nematodes can cause serious losses in yield. They reduce the shelflife of the tuber

12.1.7 The Red Ring Nematode

Rhadinaphelenchus cocophilus

Distribution - Confined to certain parts of the region

Host - Coconut palm, oil palm

Mode of Life - Migratory endoparasitic; has an insect vector

Symptoms - Red or orange-red ring of discoloured tissue 3cm wide, 2.5cm beneath the surface of the stem. Young trees are susceptible usually two years before or after bearing; yellowing followed by browning, then death of lower leaves. Trees die usually 3 - 4 months after appearance of symptoms; shedding of the flowers and green fruits may occur

Importance - Causes serious losses in production. Where the disease occurs very severe on new plantings

12.2 Control of Plant-parasitic Nematodes

Control of plant-parasitic nematodes may be achieved in several ways: cultural, use of resistant varieties, biological and chemical. Before a recommendation can be made it is imperative to note the following factors: crop type, crop age, soil type, climatic factors and qualitative and quantitative nematode populations. The recommendation should involve the use of nematicides for control only where absolutely necessary. The use of nematicides for controlling plant parasitic nematodes becomes necessary for the achievement of short-termed results. For long-termed management of plant nematode problems, a more comprehensive control strategy, emphasising cultural methods, ought to be developed. The following is a brief account of some of the methods employed in achieving nematode control that are more suitable for the region:

12.2.1 Cultural

a. Prevention of spread

Nematodes move very slowly on their own power and depend on agents for spread. They can be spread within fields, to other fields and from one country to another in various ways. The use of clean machinery, tools and reusable containers are important in avoiding short-distance spread, whereas the use of clean plant-propagules is the principle means of avoiding spread from one country to the other.

b. Fallow

This practice of keeping the land free of all vegetation by rotavation etc. results in the reduction of nematode numbers by starvation and dessication. However, in small countries with limited land resources this practice has limited application.

c. Crop Rotation

This is the most effective and widely used land-management practice. It involves the use of resistant crops between susceptible crops and results in a reduction in the nematode population density to such a level that would allow the planting of susceptible crops without suffering significant losses. Good control may be obtained by including two resistant crops between susceptible crops.

d. Use of organic amendments

The use of manure is reported to result in some measure of control of some types of nematodes under certain conditions. However, it has been observed that adverse effects on rootknot nematode populations have not been obtained when, for instance, heavy 'manuring' is used in celery production.

e. Removal of infected material

The uprooting of infected material will help to reduce nematode population densities by preventing any further reproductive activities of the nematodes and resulting in death by dessication.

f. Weed control

Several weeds are alternate hosts of plant-parasitic nematodes. Good weed control is thus essential in keeping nematode populations down to acceptable levels

12.2.2 Use of Resistance

This is potentially the most economical means of controlling plant-parasitic nematodes. Several varieties of tomato, for instance, have been developed for resistance to some species of rootknot nematodes. However, resistance is usually incomplete and, also, since the varieties have not been bred specifically for the region, some of them may not be suitable for cultivation under our conditions.

12.2.3 Biological

This is a relatively new area of research on nematode control. A number of soil organisms attack or are predacious on plant-parasitic nematodes. Whereas some success in field application of biological control has been claimed, its commercial use is still limited.

12.2.4 Chemical

The dependency on the use of nematicides for control of plant-parasitic nematodes has arisen essentially because of the absence of comprehensive programmes for control incorporating the range of cultural practices mentioned above and the relative inaccessibility to Third World countries of high technology developments.

The application of nematicides to soil result in the death of useful soil microfauna. Nematicides are generally highly toxic to wild life and some may cause contamination of water supplies. Nevertheless, their use is necessary for short-termed solutions to nematode problems affecting the region.

A recommendation involving the use of nematicides should consider time, rate, depth and method of application. Liquid and granular formulations of relatively recently developed nematicidal compounds such as carbofuran and oxamyl are available for use in controlling not only plant-parasitic nematodes but also insect pests. Nematicides may be used for disinfection of plant propagules or may be applied as pre-planting, at planting or post-planting, treatments.

13. LIST OF BASIC EQUIPMENT AND MATERIALS NEEDED FOR WORK IN PLANT NEMATOLOGY

Acid fuchsin

*Augur (soil probe)

Beakers (50, 100, 250, 500ml)

Blender

Buckets

Counting dishes (can be improvised but with great difficulty)

Extraction dishes (pie-pans can be modified easily)

Facial handkerchiefs (unscented)

Plastic bags

Formaldehyde

Glass wool/beads

Lactophenol

Microscope, dissecting (magnification range x 20 - 60)

Microscope, light compound

Nail polish

Plastic sheet

Plastic labels

Picks (can be home-made)

Scalpels or razor blades

*Sieves, series of: coarse, 100 -, 250 -, 325 - mesh per sq. in.

*Styrofoam box (ice-box)

Syracuse watch glass

*Thermometer

Triethanolamine

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*Alternative methodology not involving these available

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THE RECOGNITION AND CONTROL OF FIELD PROBLEMS CAUSED BY WEEDS

Richard A.I. Brathwaite

CONTENTS

THE RECOGNITION AND CONTROL OF FIELD PROBLEMS CAUSED BY WEEDS

by

Richard A.I. Brathwaite

1. INTRODUCTION

Characteristics and Importance of Weeds.

1.1 Definitions

There are many definitions of 'weeds'. Common definitions include:

- a plant growing where it is not desired
- a plant out of place
- an undesirable plant
- a plant with a negative value
- a plant whose virtues have not yet been discovered
- a plant whose potentials for damage outweigh its potentials for usefulness
- a plant which, if allowed to develop in the system, causes financial loss in any of many ways

All of the definitions indicate that any species at any one time can be considered a weed. For example, a corn plant in a bodie bean field will be considered a weed (volunteer corn). Nut grass in a savannah grass lawn will be considered a weed. Sensitive plant in a pangola grass paddock will be considered a weed. Consider the saying "One man's crop may be another man's weed".

Probably the best definition of weeds is as follows:

- plant species are considered weeds when they interfere with man's welfare or his activities. Such plants are undesirable because they are competitive, pernicious and persistent (Table 1).

Table 1. The world's ten worst weeds[1]

Scientific Name	Common Name
Cyperus rotundus L.	Nutgrass
Cynodon dactylon (L.) Pers.	Devil gras
Echinochloa crusgalli (L.) Beav.	Barnyard grass
Echinochloa colonum (L.) Link	Jungle rice
Eleusine indica (L.) Gaertn	Fowlfoot grass, Iron grass Yard grass
Sorghum halepense (L.) Pers.	Johnson grass
Panicum maximum Jacq.	Guinea grass
Eichhornia crassipes (Mart.) Solms	Water hyacinth
Imperata cylindrica (L.) Beauv.	Cogon grass
Lantana camara	Black or white sage

[1]Source: L. Holm (1969). Weed problems in developing countries. Weed Science 17: 115-118.

Table 2. Some common weeds associated with vegetable crops in Trinidad[1]

Scientific Name	Common Name
Cyperus rotundus	Nutgrass
Portulaca oleracea	purslane
Amaranthus spp.	Bhajee
Eleusine indica	Fowlfoot grass
Paspalum fasciculatum	Bamboo grass
Brachiaria platyphylla	Wild para
Boerhavia diffusa	Hog weed
Parthenium hysterophorus	White top

[1]Information from B.Sc. (Agric.) Part III Course 312: Project Report by Mr. C. Thomas (1981) and surveys by R.A.I. Brathwaite and S.M. Harryram.

Plant species as weeds

Some plant species occur as weeds 99% of the time. Examples of such species include *Cyperus rotundus, Paspalum fasciculatum, Parthenium hysterophorus*. Of the 200,000 species of angiosperms recorded, some 8 - 9,000 are considered weeds in agriculture (see Table 2).

1.2 Classification of Weeds

There are many different categories for classification. Weeds may be classified according to:

1.2.1 Life Span

annuals	*Cenchrus echinatus*	(Bur grass)
perennials	*Sorghum halepense*	(Johnson grass)

1.2.2 Growth Habit

vines	*Centrosema pubescens*	(Centrosema)
shrubs	*Eupatorium odoratum*	(Christmas Bush)
trees	*Psidium guajava*	(Guava)

1.2.3 Texture

herbaceous	*Achyranthes indica*	(Soldier rod)
woody	*Urena lobata*	(Cousin Mahoe)

1.2.4 Habitat

terrestrial

epiphytic

aquatic

Aquatic weeds are subdivided into

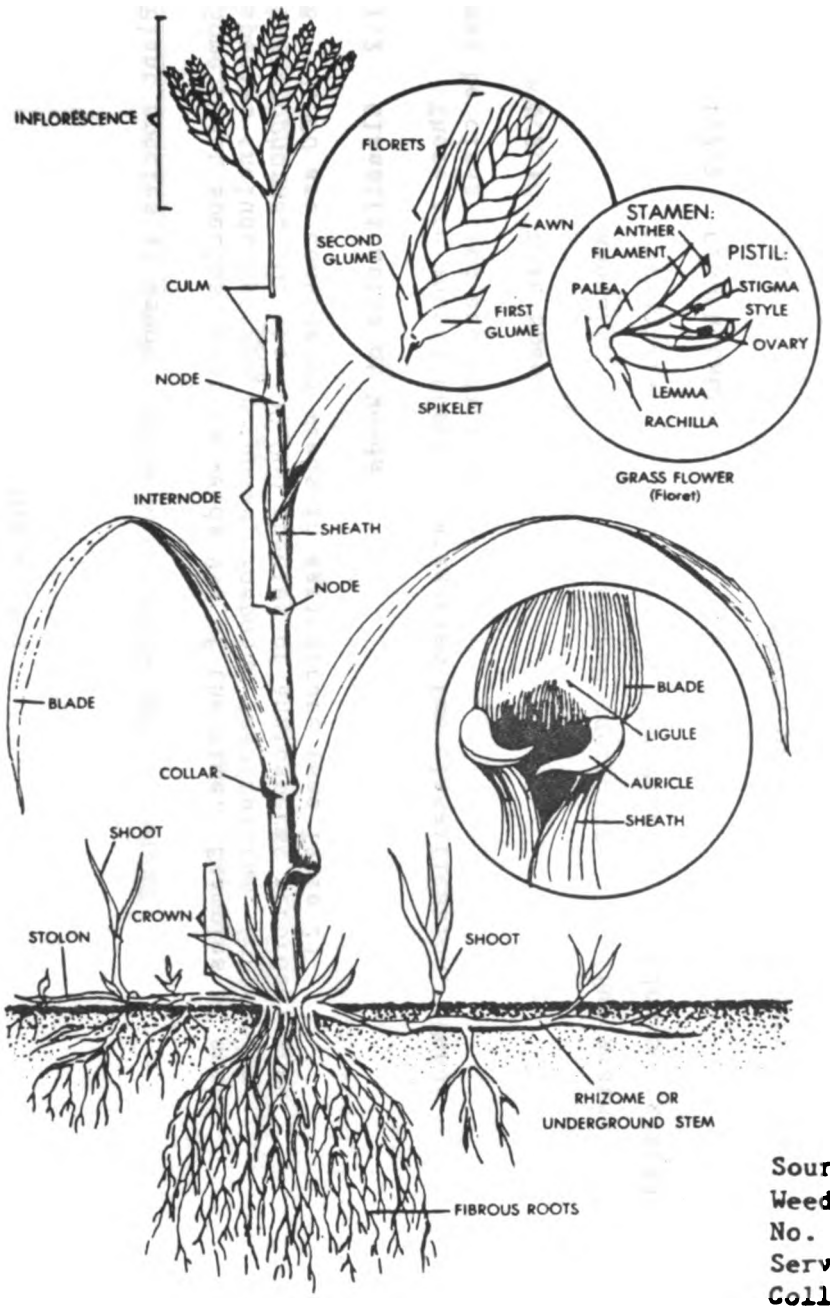
(i) emergent (upper portion above water but roots anchored to the ground)

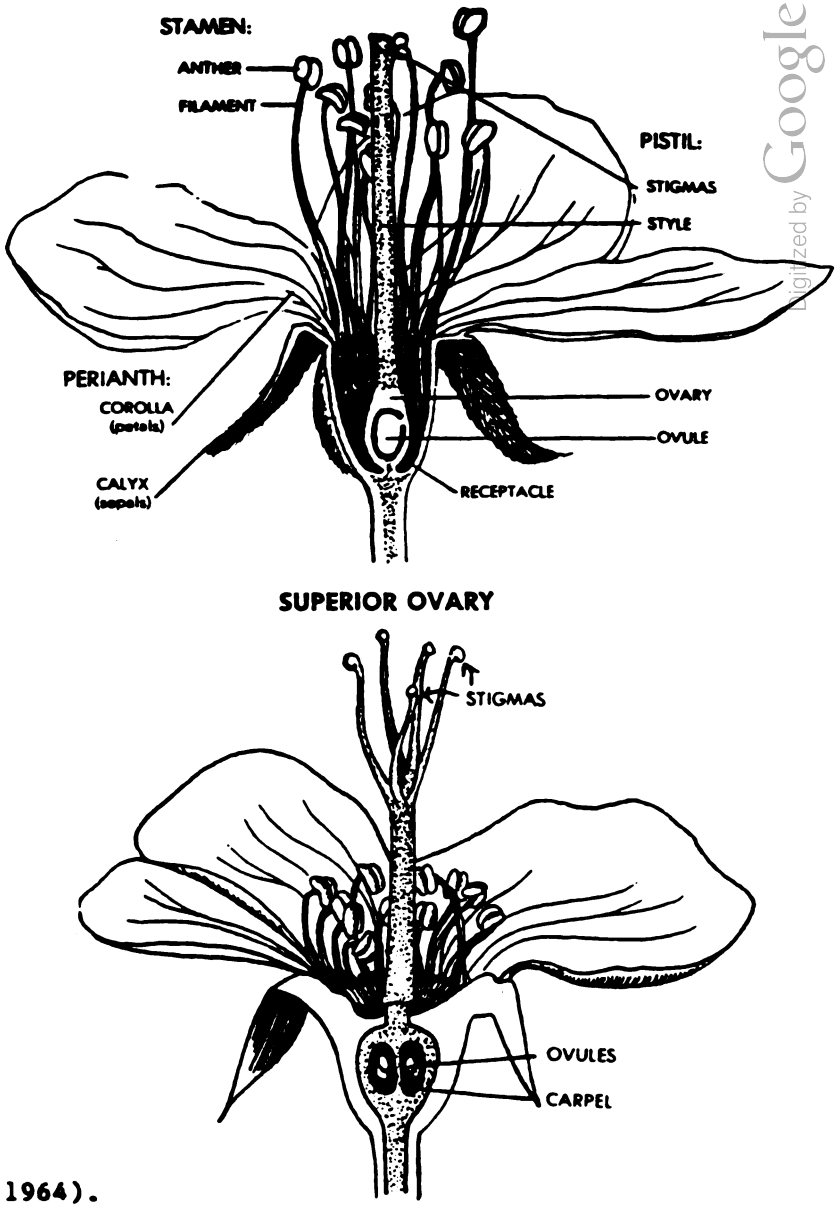
(ii) submerged (all parts are under water)

(iii) floating (upper portion above water but roots not anchored to the ground)

In Weed Science Research the following classification is used: (see Figures 1 and 2).

Fig. 2. Generalised drawing of dicotyledon plant





Source: J.F. Miller (1964). Weed identification. Bulletin No. 632. Cooperative Extension Service, University of Georgia, College of Agriculture, Georgia, USA.

1.2.5 Grasses - Family Graiminaceae

These range from small, twisted, erect or creeping annuals to perennials. Stems are usually hollow, are called culms and have well defined nodes and internodes. The leaves arise alternately in two rows from the nodes. Each leaf is composed of two parts: the sheath which clasps the stem, the margins overlapping to form a tube, and the blade which is usually thin, long, narrow and linear with parallel venation. At the junction of the blade and the sheath there is a tongue-like membraneous, often hairy, out growth called the ligule. Examples include *Eleusine indica, Echinochloa colonum, Cynodon dactylon.*

1.2.6 Sedges - Family Cyperaceae

These bear a close resemblance to the grasses and can be distinguished by their thin triangular stem, the absence of a ligule and the fusion of the leaf sheaths to form a tube around the stem. Perennial sedges have underground tubers and/or rhizomes. Examples include *Cyperus rotundus, Fimbristylis miliacea.*

1.2.7 Broad-leaved weeds

Many families of Monocotyledonae and Dicotyledonae. These are identified by their fully expanded, broad-leaved structure with netted venation. Examples include *Amaranthus spinosus, Euphorbia hirta, Cleome ciliata.*

1.3 Characteristics of Weeds

a. Successful competition for water, nutrients and light (Tables 3, 4, 5 and 6)

b. Sexual and asexual reproduction

Rottboellia exaltata can produce more than 700 tillers and branches which are all capable of flowering

c. Large number of seeds produced even under adverse conditions (Table 7)

d. Seeds of many species survive burial in the soil and undergo periods of dormancy

d. Many weed species have a variety of special adaptations for seed dispersal

f. Vegetative propagules allow species to extend itself to new sites for nutrients and water

Table 3. Effect of[1] the duration of weed control on the yield of mung bean

Duration of weed control (weeks)	Yield (kg/ha) Wet season	Dry season
0 - 1	37	142
0 - 2	354	303
0 - 3	402	1092
0 - 4	313	1019
0 - 5	1021	1258
0 - 6	898	990
0 - 7	937	1054
0 - 8	948	1283
LSD (0.05)	352.3	282.4

[1]Source: M.T. Madrid Jr. and M.R. Vega (1971). Duration of weed control and weed competition and the effect on yield. I mung bean (*Phaseolus aureus* L.) Phil. Agri. 55: 216-220.

Table 4. Effect of[1] the duration of weed competition on the yield of mung bean

Duration of weed competition (weeks)	Yield (kg/ha) Wet season	Dry season
1	840	1148
2	978	975
3	774	964
4	523	1158
5	262	746
6	132	677
7	39	566
8	8	266
LSD (0.05)	163.3	425.7

[1]Source: Madrid and Vega (1971).

Table 5. Critical periods for weed control in selected crops[1]

Crop	Period from planting (days	Days to maturity
Corn	35	120
Soyabean	40	125
Mungbean	20-35	60-65
Peanut	42	105
Okra	40	42 (1st harvest)
Onion	56	110

[1]Data from various publications by R.A.I. Brathwaite

Table 6. Comparison of yield in clean weeded and unweeded crops[1]

Crop	Yield (t/ha) Clean weeded	Unweeded
Bodie	20.1	6.4
Blackeye (dry)	3.8	0.8
Mung bean (dry)	1.2	0.3
Pigeon pea (green pods)	5.3	1.5
Corn (green)	24.4	8.2
Rice (upland)	3.5	0.4
(swampland-transplanted)	4.9	2.5
Eggplant	13.9	5.6
Cabbage	17.4	7.6

[1]Data taken from various publications by R.A.I. Brathwaite

Table 7. Seed size and number of weed seeds produced per plant in some important Caribbean weeds[1]

Weed	1000 seed weight (g)	Seeds per plant
Agemone mexicana	2.50	45 810
Amaranthus spinosus	0.46	7 810
Cleome ciliata	0.26	2 356
Cynodon dactylon	0.30	985
Digitaria sanguinalis	0.69	839
Echinochloa colonum	1.85	2 185
Eleusine indica	0.28	3 259
Euphorbia hirta	0.19	10 415
Portulaca oleracea	0.83	2 862

[1]Data from surveys conducted by R.A.I. Brathwaite and S.M. Harryram

g. Vegetative propagules allow for vigorous and faster establishment of weed species

h. Allelopathy

1.4 Losses due to Weeds

There is a general agreement that the losses caused by weeds are greater than the combined losses from diseases and insect pests. Farmers are often more concerned about the crop damage from diseases and insect pests, probably because such damage is easier to visualize unlike the subtle effects of weed growth on the crop.

1.5 Effects of weed infestation in food crops

a. Competition for

(i) light

(ii) moisture

(iii) nutrients

b. Weed control accounts for a large outlay of chemicals, equipment and man hours

c. Weeds harbour other crop pests such as insects, plant pathogens and nematodes

d. Weeds limit the choices of cultural practices and crop rotations

e. Weed contaminants cause reduction in the quantity and quality of crop harvested

f. Weeds cause losses and unthriftiness of livestock when poisonous species are consumed

g. Weeds reduce the value of livestock products

h. Weeds interfere with crop harvesting

i. Weeds reduce the real estate and aesthetic value of land

j. Weeds cause allergies and dermatological problems in man

k. Parasitic plants like love vine (*Cuscuta* spp.) and birdvine (*Phoradendron crassifolium*) are important weeds

j. Aquatic weeds clog drainage and irrigation channels.

2. DESCRIPTION OF SOME IMPORTANT WEEDS

WEEDS	DESCRIPTION
1. Ti Marie, Sensitive plant *Mimosa pudica* L. Family: Leguminosae Other species: *M. casta*	A branched perennial herb, woody at base. Semi-erect or prostrate and forming mounds, clambering 50cm or more. Stems long, hairy with abundant sharp, recurved spines, branches conspicuously angular. Leaves alternate, compound bipinnate 15-30 pairs leaflets 6-10mm long with rounded ends, tactile closing on contact or heat stimuli. Inflorescence round 1-1.5cm diameter, pink on solitary or clustered peduncles, stamens twice as many as the petals, mild fragrant odour. Pods linear to oblong, 2-5 constrictions, spiny. Seeds brown to pale brown. Reproduced from seeds and cuttings. Found in many tropical areas, commonly growing on pastures
2. Watergrass, French weed, *f. Commelina diffusa* Burm. Family: Commelinaceae Other species: *C. elegans* *C. benghalensis*	Perennial herb, succulent creeping stems, rooting at nodes, variable length 50-100cm. Leaves lanceolate or elliptic, hairy at the margin of leaf blade and top of leaf sheath. Inflorescence cymose and arise from leaf axils, is pedicelled and blue in colour. Pedicels 10-15mm long. Inflorescence enclosed in leaf-like spathe. Inner petals 6-7mm long outer ones smaller. Reproduced by creeping stems and seeds. Common weed in tropics under damp, shady conditions.
3. John bush, marsh miller, Wildhops. *Blechum pyramidatum* (Lam.) Urb. Family: Acanthaceae	Stems slender, usually short trailing 30-70cm long. Leaves pointed at tip, sparsely hairy 2-7cm long, 1-5cm broad. Small trumpet-shaped flowers, light mauve, falling easily in spikes with large leafy bracts. Fruits consist of 12 flattish seeds enclosed in bracts. Usually found growing in shady areas in the American tropics.

WEEDS	DESCRIPTION
4. Blacksage *Lantana camara* L. Family: Verbenaceae	A spreading thicket-forming aromatic shrub. Stem hairy, rough, angular sometimes prickly 1-6m or more high. Leaves opposite, ovate 3-10cm long, 2-7cm wide, accuminate, slightly toothed. Inflorescence: flowers tightly clustered into compact head 2-4cm diameter at the end of stalks 1-10cm long arising from the axils of upper leaves. Individual flowers are tubular about 1cm long with 4 or 5 irregular lobes, usually yellow turning orange to red. The fruit is an oval shaped drupe initially green turning black or purple at maturity 4-6mm in diameter usually 2 seeded and in clusters. Flowers year round. Propagates through seeds and rooting from lower branches. Common in tropical areas. A weed of plantations, pastures and waste places.
5. Callaloo, Calalu, spinach *Amaranthus dubius* Mart. Family: Amaranthaceae Other species: *A. virilis* commonly called Bhaji, Pigweed, White caterpillar; *A. spinosus* commonly called prickly calulu, prickly caterpillar or wild calalu	An erect annual herb having alternate long stalked leaves. The stems are mainly smooth and pinkish with short curled hairs. Top part of the flowering shoot is usually leafless. Flowers are very small, unisexual and borne in dense terminal and auxillary spikes. Female flowers have 5 parts, male flowers 4-5 stamens. The fruit is smooth and splits in the middle giving one dark reddish-purple seed. Propagated mainly from seeds. Usually found in the tropics mostly on arable land, pasture margins and fallowed lands.
6. Purslane, pusley, hog bhagee *Portulaca oleracea* Family: Portulacaceae	A fleshy annual, smooth throughout, forming a mat-like growth often with reddish stems 20-50cm long. Leaves small rounded at the tip, narrowing to a sessile base alternate (sometimes nearly opposite) or clustered together in whorls. Flowers produced all year and are in terminal or axillary nodes either solitary or inclusters of up to 5 subtended by 2 fleshy ribbed unequal sepals

WEEDS	DESCRIPTION
	and 5 petals. Flowers open in the morning under bright sunlight and close in the afternoon. Fruit is a capsule 6-8mm long and 3-4mm wide which splits transversely to release numerous minute oval warty black seeds. Replicates by cuttings and seed. Widely distributed throughout the tropics. Found in upland vegetable crops and uncultivated lands.
7. Coco chat, Dog teeth *Solanum stramoniifolium* Family: Solanaceae Other species: *S. Jamaicense*, *S. ficifolium* commonly called Gully bean, small red, Turleba, Susumber, Turkey berry; *S. ciliatum* -	A shrub 0.5 - 1.5m in height. Leaves are stalked. There are spines on leaves, stalks and stems. Flowers are about 2cm in diameter. Seeds are 1cm across and red when ripe. Common in uncropped lands in Trinidad and South America.
8. Duppy needles, Spanish needle, Railway daisy *Bidens pilosa* Family: Compositae	An erect branched aromatic annual herb 30-150cm tall with trifoliate leaves. The stem is square and hollow with prominent ridges green or dark red in colour. It has an opposite phyllotaxy. The highest and lowest leaves are usually simple while the intermediate ones generally have 3 or 5 leaflets with toothed margins. Leaflets are 3-7cm long and 1-2.5cm wide, ovate-shaped with sharply pointed tips. Flower heads are terminally borne on long slender stalks with yellow tubular disc florets and may have about five white florets surrounded by 2 rows or narrow bracts. Fruits are thin and long, black coloured, straight and slightly curved. Usually 1-1.5cm long with 2-4 barbed awns. The plant reproduces by seed and is a common weed of the tropics along roadsides, uncultivated and arable land.

WEEDS	DESCRIPTION
9. **Nutgrass** *Cyperus rotundus* L. **Family:** Cyperaceae Other species: *C. brerijohns* (Rotth.) Endl. ex. Hassk. *C. ferax* L.C. Rich. *C. odoratus* L. *C. sesquiflorus* (Torr.) Mattf. & Kiik *C. timifolius* (Steud.) *C. tenuis* Giv.	Rhizomatous, tuberforming perennial herb 15-50cm high with flowers in reddish brown spikelets arranged in terminal umbels. The stem is a system of spreading woody rhizomes covered in dark scales and forming black irregular-shaped tubers 1-2cm long. Above-ground stems are erect, smooth, 3-angled and swollen at the base. All leaves are basal and narrow about 5mm wide, normally shorter than the flowering stem with cylindrical sheaths at the base. Inflorescence borne by several small leafy bracts with main rays varying between 2-6cm in length and spreading groups of 3-8 spikelets towards the tips. Spikelets are usually 1-2cm long, 2mm wide flattened and acute at the tip turning reddish brown at maturity and consisting of 10-30 closely overlapping florets. The outer scales are 3mm long and blunt tipped. Seeds are triangular-shaped and blackish when ripe. The plant reproduces by rhizomes, tubers and seeds. It is generally regarded as the world's most troublesome weed and is distributed throughout the tropics and subtropics. Common in gardens, lawns, roadsides, open areas.
10. **Devil grass, Bahamas Grass, Bermuda grass** *Cynodon dactylon* (L.) Pers. **Family:** Poaceae (grasses)	A perennial with branched creeping stems, rhizomes and surface stolons. Roots and tufted shoots grow from the nodes. Leaf blades are flat and light to bluish green to 5cm long, sometimes longer and 1.5-5mm wide tapering from the base to tip. Hairless sheaths overlap on aerial stems except for a ring of short hairs forming a ligule at base of leaf blade. Inflorescence consists of a flowering head 3-5 spikes usually on a single whorl. Spikelets are sessile flattened and 2mm long arranged in 2 alternate overlapping rows along the underside of the spikes. They are awnless with one

WEEDS	DESCRIPTION
	fertile floret. The plant is propagated by seeds but mainly through runners and rhizomes. It is widespread throughout the tropics, common in pastures and stony waste lands, can be a persistent weed of arable crops. It is used as a lawn grass.
11. Fowl foot grass, Yard grass, Goose grass, Iron grass, Dutch grass *Eleusine indica* (L.) Gaertn. Family: Gramineae-Chlorideae	It is an annual or perennial grass growing in tufts and may be erect and prostrate. It has glabrous, very tough shoots. The stems are flattened and rise to 25-60cm. Leaf blade and sheaths are prominently keeled and very glossy with membranous ligules. Inflorescence arises in a terminal whorl of 2-6 spikes, 4-8cm long or more and 3-6mm wide; often with one or two additional spikes slightly below the others. The spikelets run in two rows along one side of the rachis. Seeds about 1mm long, reddish brown in colour with outstanding ridges. The plant replicates by seed and is widely distributed throughout the tropics. Is a common weed of pastures, lawns, cultivated and uncropped lands.
12. Seed under leaf, carry-me-seed, Chamberbitter, Egg woman, Gripe weed. *Phyllanthus amarus* Schum. Family: Euphorbiaceae Other Species: *P. amarus* - whole plant usually green *P. urinaria*- More spreading plant, stems reddish	A tall thin, much-branched erect annual herb. May be 12-80cm tall. The stem is smooth and woody at the base. The leaves are pinnate with numerous small elliptic to oblong - shaped leaflets 5-8mm long and 3-4mm wide. The leaflets are alternatively arranged on the rachis. Inflorescence-flowers are whitish or pale green and produced in angle of leaves. Fruits are small, smooth, brown, rounded capsules, 1.5-2mm in diameter with 6 minute seeds. The plant is propagated by seed and is generally distributed throughout the tropics. It is usually found growing in both cultivated and waste land

WEEDS	DESCRIPTION
13. Gamalot *Setaria poiretiana* (Shult.) Kunth. Family: Graminaceae - Panicaleae Other species: *S. paniculifera* (Steud.) Fourn. *S. barbata* (Lam.) Kunth	A large perennial which grows in tufts 1-2m tall. The blades are coarse with pronounced ridging up to 1m long and 10cm wide. The leaf sheaths and ligules are hairy. The panicle is profusely flowered on a long stalk 60cm in length and 10cm in width. There are many branches which droop to one side. It is a weed commonly found in Mexico, Brazil, and in Trinidad in citrus and cocoa farms.
14. Poppy *Argemone mexicana* L. Family: Papaveraceae	An erect branched hairless annual about 1m tall and has prickly blue green leaves with showing yellow flowers. The stem has loosely distributed prickles and exudes a yellow juice when cut. The leaves are alternate, sessile, clinging to the stem at the base. They measure up to 15m long and 5cm wide, are deeply lobed, irregularly indented with sharp spiny margins and whitish veins. Branches terminate in a single flower 4-5cm in diameter and consist of 3 prickly sepals, 6 bright yellow petals and a large number of stamens. The fruit is a spiny capsule 2.5cm long with 4-6 divisions opening by slits at the tip. Seeds are round, black and about 1.5mm across. Propagated by seed. Mainly weed of roadside and waste lands.
15. Milkweed *Euphorbia heterophylla* L. Family: Euphorbiaceae Other species: *E. cyathophora* Murr. *E. oerstediana* (Klotzsch & Garcke) Boiss	An annual herb usually unbranched and erect attaining a height of 30-80cm. Produces white latex. Leaves may be simple or lobed and concentrated towards the top of the stem. The stem is hollow, round, often with hairs. Leaves are variable in shape oblong - lanceolate to ovate, 6-15cm long and 4-7cm wide. Lower leaves often have a reddish stalk, 1-2cm long. Lamina - simple and pointed at the tip, with toothed or sinuated margin. Leaves higher up on the stem are sessile with one or two pairs

WEEDS	DESCRIPTION
	of slightly indented lobes and blunt tips. The inflorescence consists of clusters of a large number of short pedicels without petals and sepals but has conspicuous glands surrounded by radiating leaflike bracts. The fruit is a hard 3-lobed capsule with reddish blotches and contains 3 seeds. Reproduces by seed and is common in the tropics where it is usually found both in cultivated and uncultivated areas.
16. Hay grass, wire grass, tapia grass *Sporobolus indicus* L. R. Br. Family: Gramineae - Agrostideae Other species: *S. jacquemontii* Kunth	Tufted perennial with wiry stems, 60cm tall during flowering. Leaf blades 10-30cm in length, 3mm wide. Hairless. The ligule is a fringe of hairs. There are a large number of small spikelets in an erect panicle with several branches. Spikelets are small and torpedo-shaped, one seeded. Seeds are approx. 2mm long. Common in pastures and waste places throughout the tropics.
17. Cerasee Buse, Carilla, Maiden's bush, Miraculous vine *Momordica charantia* L. Family: Cucurbitaceae	A slender branched vine with tendrils. Phyllotaxy is alternate. Leaves, are thin, strongly scented, deeply indented 5-7 lobes. Flowers are yellow. Fruits are lantern-shaped and suspended 4-6cm long, fleshy, bright orange, eventually splitting open to expose seeds covered with bright red coloured pulp. Widely distributed throughout the tropics in thickets, hedgerows, beaches, fences and trailing over grass.
18. Crab grass *Digitaria adscendens* (Kunth) Henrard Family: Gramineae - Paniceae Other species: *D. horizontalis* commonly called Finger grass *D. cilionis* (Retz.) Koeler	An annual with the lower parts of the stem prostrate. Flowering parts rise to as much as 75cm but may usually be shorter. Leaf sheaths are bristly with long hairs and keeled. Leaf blades are rolled in a bud and slightly keeled, being broadest towards the base, usually hairy beneath but not more than 5-15cm long and about 1cm wide. There is a brown membranous ligule, 2-3mm long. Spikes of flowers maybe in one or two whorls 5-10cm long. Spikelets 3mm long.

WEEDS	DESCRIPTION
	Pantropical weed occurring in arable land, pastures and stony uncropped waste lands.
19. Bamboo grass, bull grass *Panicum fasciculatum* Sw. Family: Gramineae-Paniceae Other species: *P. maximum*	Robust tufted annual with loose spreading shoots 50cm tall at flowering. Leaf blades are hairless but upper surface and leaf margins rough, shiny and light green 10-30cm long, 1-3cm wide. Ligule consists of hairs 1mm or more long. Few to many golden brown to chubby spikelets arranged on one side of each branch make up the inflorescence (plants from Jamaica do not have long hairs accompanying the spikelets). Common in pastures, arable as well as roadside lands in the tropics and citrus orchards.
20. Purple top *Chloris barbata* Sw. Family: Gramineae-Chlorideae Other species: *C. radiata* (L.) Sw. commonly called Pale Finger Grass	Tufted annual rooting freely from the lower nodes usually 30-50cm tall. Leaf blade narrow, pointed and about 20cm in length, enclosed in bud and keeled. Leaf sheaths are keeled, tightly clasping and hairless. 7-10 spikes arise from the same terminal point, 4-6cm long. On each spikelet there are 3 awned florets arranged in two rows on one side of the spike. They are initially purple turning black. The larger sterile floret is broad at the top. Occasionally on arable land but more common in dry uncultivated areas throughout the tropics.
21. Lion's tail, Candle stick Chandelier, Bald bush, Man Piaba *Leonotis nepetifolia* (L.) Air. f. Family: Labiataceae	An erect annual herb 1-1.5cm high with a 4-angled stem. Leaves are stalked, toothed and mostly 10cm long. Flowers are orange, tubular and borne in dense clusters located at the top of the shoot. Prevalent on arable land and waste places in the tropics.

WEEDS	DESCRIPTION
22. Para grass *Panicum muticum* Forsk. Family: Gramineae-Paniceae Other species: *P. pilosum*	A perennial with creeping and rooting, long lower stems. Flowering shoots are 1-3m in height with pubescent leaf sheaths and even more hairy nodes. Leaf blades are 10-15mm in width, keeled, with pointed tips, soft and no hairs. The ligule is membranous and with vestigial hairs 1-15mm long. Spikelets are 3mm long and arranged on one side of the flowering branches. Commonly found in ditches, swamps and river banks in the tropics.
23. Wildslips, wild potato *Ipomoea tiliacea* (Willd.) Choisy Family: Convolvulaceae Other species: *I. hederifolia* L. *I. nil* (L.) Poth	A twining vine with long thin stems releasing a thin milk sap when broken. Leaves are stalked alternate, broadly ovate or rarely 3-lobed. May be 4-12cm long and 3-8cm in width. Flowers are bell-shaped, may be more than one together, variable in colour from light pink to rich purple, usually darker in the centre. Fruit is a rounded capsule with dark brown to black seeds. May be found widespread on fences, hedgerows and thickets in the tropics.
24. Consumption weed *Cleome ciliata* Schum. & Thonn. Family: Capparaceae Other species: *C. viscosa* L. *C. aculeata* L. *C. spinosa* Jacq. *C. aculeata* has slightly larger leaflets and prickle at the leaf base *C. spinosa* has distinctly spiny stems and numerous flowers with white or pink petals	An annual herb with alternate leaf arrangement. The leaves are compound trifoliate on stalks 1-5cm in length. The leaflets are sparsely hairy, ovate - lanceolate with a sharp narrow tip and tapering base. Inflorescence arises in the upper leaf axils on slender stalks 3cm long. Flowers are white or mauve. Fruits are green pods cylindrical and narrowed at both ends up to 9cm long, 3-4mm wide on stalks 1.5-2.5cm long splitting into 2 halves from below. They are seed propagated. Common in open waste lands in the tropics, paddy fields and upland crop fields.

WEEDS	DESCRIPTION
C. viscosa has yellow flowers and the plant is almost entirely covered with sticky glandular hairs	
25. Savannah grass, carpet grass flat grass, broadleaf grass *Axonopus compressus* (Swartz) Beauv Family: Gramineae-Paniceae	Creeping perennial with flattened hairy shoots 30cm tall. The leaf blade is brilliant green with crinkled pubescent margin and narrowly rounded tip 5-20cm long and 1cm wide. There is a vestigial ligule with prominent keel, tightly clasping leaf sheaths, open at the throat and usually purplish coloured. Spikelets 2-3mm long. Common in lawns, damp pastures and shady places in the tropics.
26. White top, white head, barley flower, bastard feverfew mugwort, wormwood *Parthenium hysterophorus* L. Family: Compositae	An annual aromatic herb 120cm high, with alternate deeply dissected leaves 11cm long and 6cm broad. Hairy leaves, numerous rather small flower heads in a much-branched white-coloured inflorescence 4-8mm broad. Black, flat seedlike fruits 2mm long with 2 recurved bristles. Common in waste places and arable land.
27. Wild or white para *Brachiaria cruciformis* (Sm.) Griseb. Family: Gramineae-Paniceae Other species: *B. extensa*	An annual growing in tufts and rooting at the lower nodes. May be 30cm in height. Stems and leaves are smooth or sparsely haired. Ligule is a fringe of hair 1mm long. Leaf blade 4-6cm in length and 8mm in width. There are about 7 spikes close together 5-20mm long. Hairy spikelets 1.5mm long. Common weed in Barbados cane fields and Antigua.
28. Rabbit vine, sweet pinder *Teramnus labialis* (L.f.) Spreng. Family: Leguminosae-Papilionaceae	Slender twining vine with alternate leaves consisting of three leaflets, thiny haired underneath. Leaflets ovate to elliptical, pointed at the tip 5.5cm long, 3cm in width. Small pinkish flower. Tiny hairy pod 2.5-5cm long with short terminal beak, abruptly upturned. Common on stony waste land and thickets in tropics.

WEEDS	DESCRIPTION
29. Corn grass *Rottboellia exaltata* (L.) L.f. Family: Gramineae-Andropogoneae	Robust, erect or straggling annual, 3.5m tall with long narrow spikes and hairy stems causing irritation to skin when touched. Stems are stout with stilt roots at the base. Leaves are pale green and hairy with white midrib and sharp edges. The blade tapers at both ends, 60cm long and 2.5cm wide. Stiff, brittle irritating hairs are borne mainly on sheaths of the lower leaves. Inflorescence contains cylindrical spikes 8-15cm long, 3-4mm thick, borne singly in upper leaf axils, stalks partly enclosed in leaf sheaths. Spikelets are 4-5mm long, paired, awnless, one of each pair sessile, yellowish, hard and lying in a depression of the axis. Upper spikelets sterile, seed cylindrical with segments of axis and seed attached. Seed propagated, common on arable and uncultivated lands.
30. Seymour grass *Andropogon pertusus* (L.) Willd. Family: Gramineae-Andropogoneae Other species: *A. glomeratus*	Perennial with erect flowering up to 60cm tall. Long, prostrate shoots are produced in wet weather. Leaves narrow 10-20cm long, 1-4mm wide with variable hair covering. Spikes arise close together 3-6cm long. Spikelets have rough awns with a deep pit on the back of each. Among commonest grasses in drier parts of southern Jamaica. Scattered occurrence in other areas of the West Indies.
31. Bur grass *Cenchus echinatus* L. Family: Gramineae-Paniceae	Erect, tufted annual reaching 50cm high. Decumbent at base. Leaf blades flat, thin 20cm long, 8mm wide. Spikes on long terminal stalks 7cm long. The burs are loosely packed usually purplish and broadly rounded with a ring of fine bristles at the base below the sharp spreading spines. Usually 6mm in diameter excluding the spines. Common on waste ground, open stony or sandy fields in tropics.

WEEDS	DESCRIPTION
32. Jungle rice *Echinochloa colonum* (L.) Link Family: Gramineae-Paniceae Other species: *E. crus-pavonis* (Kunth) Schult.	Annual having spreading and ascending shoots 50cm or more in height during flowering. Leaf blades and sheaths are keeled and hairless and ligules absent. Leaves 25cm long 3-7mm wide, often tinged purple. Inforescence green to purplish, 6-12cm long, 4-8 simple branches 1-3cm long, 3-4mm wide and spaced 0.5-1.5cm apart. Spikelets - all similar 2-3mm long and closely crowded in 4 rows along one side of the branch somtimes with a short awn point 1mm long. Reproduces by seed. Found all through the tropics in swampy areas, irrigated rice and upland crops.
33. Hog weeds, sow meat *Boerhavia diffusa* L. Family: Nystaginaceae Other species: *B. coccinea* Mill. *B. erecta* L.	Perennial herb, stems branched at base radiating up to 1m long. Patches of stalked glands may be present. Leaves and stems light green. Flowers very small and rich crimson in heads at the tips of slender stalks. Fruits minute, top shaped, extremely sticky and adhere to any passing object. Seed propagated. Pantropical and found commonly on waste and arable lands.
34. Guinea grass *Panicum maximum* Jacq. Family: Gramineae-Paniceae Other species: *P. fasciculatum* Sw.	A robust, tufted perennial with many loose spreading shoots, an open conical branched flowering head or panicle with a central upright stem from which long, thin more or less horizontal branches, the lowest whorled and up to 35cm long, the upper ones shorter and irregularly spaced. Spikelets are 3-4mm long, awn-less with on fertile floret, lower scales thin and green, seed light brown 2.8 x 1mm. Leaf blade linear lanceolatle 30-80cm long, 4-5cm wide, strongly keeled with a white midrib and rough margins. On the upper surface there is a dense patch of long hairs just above the membranous ciliate ligule. Common throughout the tropics in pasture margins and low dampish areas.

WEEDS	DESCRIPTION
35. Velvet bush *Lagascea mollis* Cav. Family: Compositae	An annual herb with week clambering branches growing to 1m tall. Leaf arrangement is alternate. Leaves are ovate pubescent 2-6cm long, 1-4cm broad. Flower heads are closely packed 1-1.5cm broad on rather long stalks. Very small florets white or pale mauve. Fruits 3mm long. Occurs in many islands along roadsides and waste places.
36. Scorpion weed *Heliotropium indicum* L. Family: Boraginaceae Other species: *H. angiospermum* Murr. *H. procumbens* Mill.	An erect branched, hairy herb up to 1mm in height. Leaves alternately arranged with slightly winged stalk. Blade ovate to oblong-ovate, 4-1.2cm long, 2-5cm wide, hairy, margin irregularly indented. Inflorescence - closely packed rows of small, light, violet flowers on the upper side of spikes 20cm long and curled backwards at the tip. Individual flowers-tubular, 4mm long. Fruits are in clusters of 4 nutlets. Propagated by seed. A common weed throughout the tropics on arable and open waste land and fallows.
37. Ballier savanne *Sida acuta* Burm. f. Family: Malvaceae Other species: *S. rhombifolia* L. *S. glomerata* Cav. *S. spinosa* L.	Perennial shrub, stems erect and branched. Plant is 50-150cm tall. Leaves rhomboid, pointed, sharply toothed, pubescent, linear stipule. Flowers axillary singly or in pairs, corolla yellow or fading to yellow from white with a yellow eye. Fruits have 2 sharply pointed awns. Common on waste lands throughout the tropics.
38. Johnson grass *Sorghum halepense* (L.) Pers. Family: Gramineae-Andropogoneae	A perennial growing in tufts with scaly rhizomes with erect or prostrate shoots 1.5m tall at flowering. There are a large number of suspended flowers in open inflorescence. Common in pastures and stony waste land.

WEEDS	DESCRIPTION
39. Sweetheart, feverweed, iron vine *Desmodium canum* J.F. Gmel.) Schinz & Thell Family: Leguminosae-Papilionaceae Other species: *D. adscendens* (Sw.) DC. *D. triflorum* (L.) DC. *D. tortuosum* (Sw.) DC.	Shrubby, herbaceous plant, 60cm high. Leaflets - blunt-tipped, 7cm long, greyish beneath. Flowers pink, drying bluish, pods have 3-7 segments with hooked pubescence. Common in pasture and grassy roadside banks in the tropics.
40. Vervain, verbina vervine *Stachytarpheta jamaicensis* (L.) Vahl Family: Verbenaceae	Straggling herb growing up to 1.5m high with opposite toothed leaves 2-6cm long and 1-3.5cm wide, rounded at the tip. Inflorescence is a terminal spike, 20cm long with numerous closely spaced sessile rich purplish blue flowers arising from hollows in the swollen axis, subtended by narrow pointed bracts. Corolla tubular 4-5mm long, expanded to 5 unequally rounded lobes at tip. Fruit are dark brown, oblong, 3mm splitting into 2 seeds. Seed propagated. Common in damp, waste places and cultivated areas in the tropics
41. Iron weed, inflammation bush *Vernonia cinerea* (L.) Less. Family: Compositae	An erect, hairy, slightly branched herb, 30-100cm tall. The stem is ribbed, with a few branches having thin, white hairs. Leaves are ovate, 2-8cm long, 2-3cm wide, with pointed tips and irregularly indented margins. Tapers at the base to a short winged stalk. There are many flowers in a much branched inflorescence. Florets are bright mauve and tubular, about 5mm long, with a pappus of white hairs. Plant is seed propagated. Common in waste places and rough pastures throughout the tropics.

3. DEVELOPING A WEED CONTROL PROGRAMME

There is no one weed control programme which will be suitable for all cropping situations. A well planned and properly executed programme consists of a number of appropriate techniques sequentially coordinated right from the first cultural operation for the establishment of the crop till the final harvest of the crop. Such a programme should include the following steps:

1. Diagnosis of the weed problem
2. Assessment of the available control methods
3. Selection of appropriate weed control practices
4. Execution of the programme
5. Evaluation of the programme

The following are the major points to be considered in each step:

3.1 Diagnosis of Weed Problem

A. Weeds

(i) Identification of weeds - all weeds especially new ones, should be properly and correctly identified. Keys, flora, and manuals, where available, are useful tools. You will have opportunity to use weed identification guides. Remember that it may be necessary to collect specimens for dispatch to a Herbarium for the confirmation of identification

(ii) Density of weeds - The relative economic importance and density of all species should be determined and prioritized.

B. Soil and environmental factors

(i) Information on the following soil characteristics should be collected; texture, structure, PH, contents of clay, organic matter and moisture, erosivity, surface uneveness and types and quantity of plant residues.

(ii) Wind velocities, rainfall patterns, humidity data, location of non-target species and sites should be taken into consideration

C. Crop management system

Cultural practices and cropping systems information should be considered.

D. Past experiences

3.2 Assessment of the available control methods

All available information on the different methods should be assessed under each of the following head:

(i) effectiveness

(ii) consistency

(iii) compatability with other aspects of the crop management system

(iv) flexibility

3.3 Selection of appropriate weed control practices

Aim for a weed control programme that is effective, economical and flexible and based on careful consideration of the following aspects:

a. Economics

b. The value of early season programming

c. Management system including optimum use of time, suitability to crop and management system, operational capability

d. Follow-up

3.4 Execution of the programme

Three factors are essential if the programme is to be a success. These are: operations must be carried out at the right time, proper equipment must be employed, and the equipment must be correctly maintained, adjusted and operated.

3.5 Evaluation of the programme

The programme should be evaluated to identify its success or failure both in technological and economic terms.

4. HERBICIDE NON-PERFORMANCE AND CROP INJURY

Herbicides can contribute to the development of an effective weed control programme. However problems with herbicide use do occur in some situations. These include damage to non-target vegetation and sites, lack of weed control, injury to the present target crop and subsequent crops and sick or dead animals. One or more of the following factors may cause the herbicide problem: Incorrect diagnosis of the weed problem, incorrect selection of the

herbicides, the use of improper application techniques and adverse weather conditions.

In the diagnosis of a problem of herbicide non-performance and crop injury the following steps should be undertaken:

a. Collection of background information (Table 8)

b. Assessment of lack of control or injury pattern in the field (Figure 3)

c. Assessment of injury pattern in individual plant (Table 9)

d. Interpretation of findings (Table 10).

Table 8. Information to be collected during an on-site investigation on herbicide non-performance or crop injury*

1. Locate and determine the pattern of crop injury (or herbicide non-performance) in the field. Make a map of the area.

2. Assess the crop condition. Take stand counts, record injury symptoms, and take photographs of the general area as well as close-ups of plants. Collect plants with a spectrum of symptoms (from injured to uninjured plants) for later study. Be sure to collect shoots, roots or other underground structures as well as soil in case analysis will need to be conducted.

3. Determine the herbicide used, application rate and method of application.

c. Record all of the following important information:

   (i) Dates: of seed-bed preparation, herbicide application, planting, etc.

   (ii) Weather: before, during, and following herbicide application or planting, including air temperatures, rainfall, and wind.

   (iii) Soil conditions: moisture and condition (roughness, debris, cloddiness) at planting, at herbicide application and at time of field investigation.

   (iv) Soil parameters: texture, organic matter, variability, residues, other special problems, e.g. sites of erosion. If soil samples are taken, label them with site, depth, and date of collection. Place any samples (particularly those for chemical analysis) on ice immediately.

   (v) Previous crop: herbicides used and their rates of application, lime and fertilizer rates, yields, weeds present (predominant weeds).

   (vi) Seed-bed preparation and tillage: how, when, with what equipment, and under what conditions were these operations conducted?

   (vii) Herbicide application: equipment, speed, pressure, nozzle-type operator, amount of herbicide per tank, amount of herbicide per treated area, carriers (if water, indicate source e.g. river, stream, and quality) and additives. Ask to examine the sprayer and, if used, the incorporation equipment. Enquire if the sprayer, lance and nozzle were cleaned before use for application of herbicide and if so how it was done.

(viii) Manures and fertilizer application and dates: equipment, methods of application, type of manure and fertilizer, rates of application.

(ix) Operations on adjacent areas: herbicide applications at about the same time the problem being investigated occurred. Find out the types of herbicides used, rates and methods of application, time of day when applied, wind direction at time of application.

(x) Weed species: if in doubt, collect plants for positive identification. Dig for perennial structures

(xi) Weed flora and abundance of major weeds.

(xii) Weed size: at the time of treatment, tillage, planting, etc. Stage of maturity and vigour.

5. Determine the number of other complaints and settlements the parties have been involved in previously.

*Adapted from M.A. Ross and C.A. Lembi (1985), Applied Weed Science. Burgess Publishing Company, Minneapolis, Minnesota, USA.

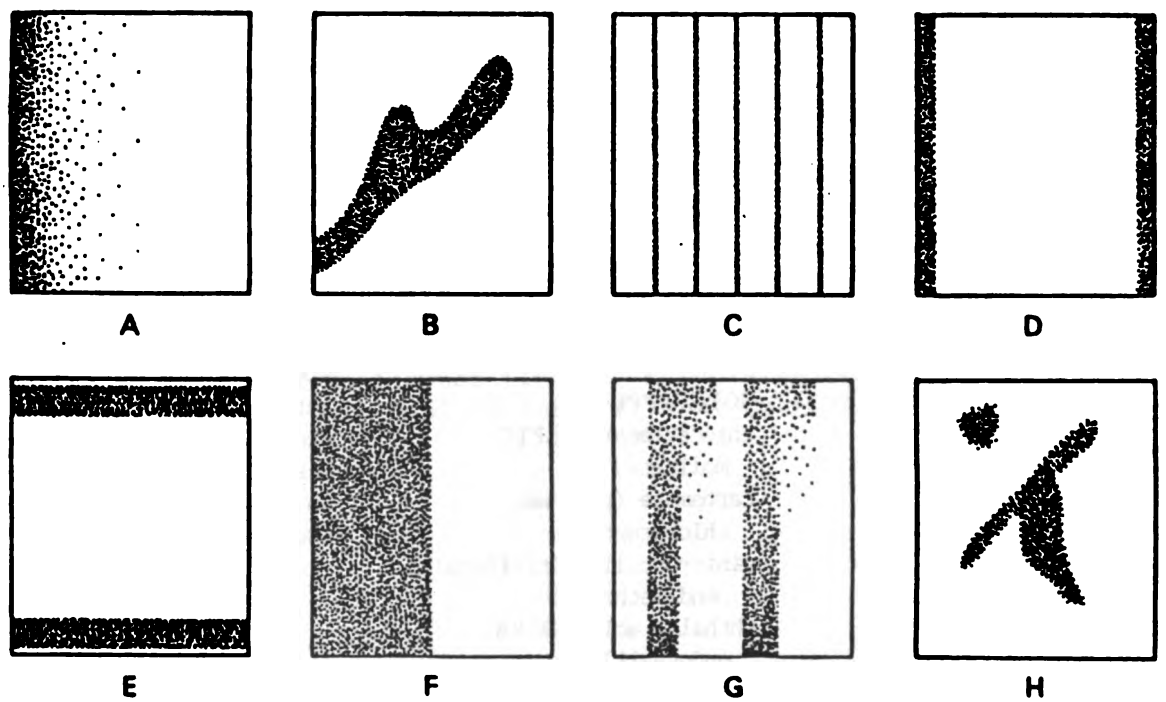


Figure 3. Illustrations of various types of field patterns of crop injury or nonperformance: (A) herbicide drift, (B) volatilization and vapor movement of herbicide into low lying areas, (C) crop injury in strips the width of equipment, indicating overlapping spray pattern or worn nozzles, (D) poor control at edges of field due to light application from outside nozzle (common with normal flood setup), (E) crop injury at ends of field due to double application, (F) definite break between normal and uninjured parts of the field, (G) strips of injury due to poor mixing or inadequate spray tank agitation, and (H) spots of injury (or lack of weed control) due to soil variables.

SOURCE: M.A. Ross and C.A. Lambi (1985)

Table 9. Classification of plant symptoms associated with herbicides

SYMPTOM	HERBICIDE GROUP	COMMENTS
CHLOROSIS: abnormal yellowing of green plant tissue; usually starts in the leaves, and as chlorosis increases, necrosis or death of the yellowed plant tissue can follow.	FOLIAR APPLIED: amitrole dalapon glyphosate DSMA, MSMA	Foliar-applied herbicides are absorbed and translocated through plant tissue and can cause destruction or interference with the photosynthetic mechanisms
	SOIL APPLIED: triazines (metribuzin, simazine) substituted ureas (linuron, diuron) uracils (terbacil)	These soil-applied herbicides move into the plant with the transpiration stream and concentrate in the leaves where they affect the photosynthetic mechanisms of the plant and cause yellowing and eventually necrosis of the tissue.
STUNTING: a reduced growth in plants. Plants do not germinate or show poor growth. Often root systems will be dramatically reduced.	FOLIAR APPLIED: carbamate (phenmedipham barban)	These herbicides produce stunting or complete lack of germination in susceptible species as a result of interference with cell division or elongation, or affect the plants' metabolic processes in such a way as to eventually cause reduced growth.
	SOIL APPLIED: thiocarbamate (EPTC, molinate) carbamate (propham, chlorpropham) dinitroaniline (trifluralin, pendimethalin) phthalic acid (DCPA, endothall) amides (pronamide, alachlor)	
DESICCATION OF PLANT TISSUE: a collapse of plant tissue at or very near the point of application. The tissue desiccates soon after application. If application is light, e.g., a few drops on a leaf, symptoms may be less severe, resulting only in necrotic spots at the point of application.	FOLIAR APPLIED: sulfuric acid weed oils dinitrophenols (dinoseb) bipyridylums (paraquat, diquat) benzonitrile (bromoxynil) diphenylether (oxyfluorfen)	These herbicides do not mvoe much within plants; they have their effects at the site of application. High dosage rates affect cell-membrane integrity, causing a leakage of materials from various organelles and a rapid desiccation of tissue. The bipyridylums affect electron transport in photosynthesis. The dinitrophenols act as uncoupling agents in respiration.

MALFORMATION OF STEMS AND LEAVES: Symptoms include elongation and cupping of leaves. Twisting of stems and petioles is also common. Stems may show swelling and splitting near the soil line. Grasses may show reduced growth, lodging, or multiple heading.	FOLIAR APPLIE: phenoxy herbicides (2,4-D) benzoic acid picloram glyphosate triclopyr	A common effect is on DNA and RNA, affecting both cell division and protein synthesis. Many of theses herbicides are referred to as 'hormone' or 'hormonal' herbicides in that they produce symptoms at very low rates of application. Drift injury is common with phenoxy herbicides.
	SOIL APPLIED: DCPA thiocarbamates (EPTC, pebulate) benzoic acid picloram triclopyr	Glyphosate can result in the production of multiple growing points at shoot tips subsequent to sublethal dosages. DCPA and the thiocarbamates result in stunting and a less severe malformation of the leaves. The thiocarbamates often cause leaves to stick together.

Source: California Weed Conference (1985). Principles of Weed Control in California Thompson Publications, Fresno, California, USA.

Table 10. Possible causes of herbicide nonperformance or crop injury

Herbicide nonperformance

Resistant weed species.

Weed at incorrect stage when sprayed.

Weed under stress at time herbicide is applied.

Environmental conditions affected herbicide performance (cold temperatures prevent activity: low humidity with foliar applications).

No rain after application of soil applied herbicide.

Excessive rain caused herbicide to leach out of weed seed zone.

Rain washed off foliar applied herbicides.

No wetting agent added to foliar applied herbicide solution.

Poor coverage with a contact herbicide.

Rates improper for soil type.

Used a lower rate than suggested.

Improper application or poorly calibrated equipment

Picked a herbicide that is inconsistent in performance for the weed species present.

Loss of activity due to antagonism with another chemical.

Weeds had time to recover after application of shortlived herbicide.

Late weed germination after herbicide effectiveness dissipated.

Used slow acting herbicide for which symptoms not yet obvious.

Given evidence available, reason cannot be determined.

Herbicide crop injury

Drift during application (or volatilization and then drift of vapors).

Too high a rate applied.

Herbicide applied at improper stage of crop growth.

Foliar applications made in liquid fertilizer, crop oil, or some other chemical when they should not have been.

Weather resulted in higher than normal injury to crop (high temperatures and humidity with foliar applied herbicides; cool, cloudy, wet conditions with soil applied herbicides).

Another problem such as insect, insecticide, or disease damage that, when added to the herbicide injury, resulted in considerably greater crop damage than any single factor alone.

Crop variety susceptible to herbicide.

Herbicide dependent on depth placement for selectivity placed too near or on the seed.

Excessive rain on a normally immobile herbicide.

* Adapted from M.A. Ross and C.A. Lembi (1985).

RECOGNITION OF SYMPTOMS OF NUTRIENT DISORDERS AND THEIR METHODS OF CONTROL

Selwyn M. Griffith

CONTENTS

RECOGNITION OF SYMPTOMS OF NUTRIENT DISORDERS AND THEIR METHODS OF CONTROL

by

Selwyn M. Griffith

1. INTRODUCTION

Recognition and knowledge of the specific plant symptoms for the various nutrient disorders is often of considerable value in the diagnosis of nutritional problems and has provided field workers, extension officers, students and research scientists in subtropical and tropical countries with an invaluable preliminary diagnostic aid. To a large extent it eliminates guesswork and sharply focusses on the direction in which further investigation of the problem should proceed. If the recognition is done satisfactorily by an experienced investigator it will reduce the necessity of conducting numerous and costly crop - fertilization experiments to gain information about the nutrient element requirements of crops.

From a physiological standpoint a nutrient deficiency could be described as that point at which the physiological development of the plant has been impaired to the extent that different visual symptoms, which possibly could be considered abnormal, could be discerned. This definition makes no allowances for a lack of growth and/or a decrease in yield that may occur as a result of the plant getting less than its requirements. However, nutrient element levels which range just above deficiency and at which adquate yields are not obtained yet nothing shows up on the plant, are recognised and termed areas of hidden hunger.

For individual nutrient elements there are threshold values which invariably differ from site to site. Crops unable to absorb sufficient nutrients to satisfy their minimum requirements, will develop an abnormal appearance, which is referred to as a nutrient disorder (Ishizua, 1971). Symptoms of nutrient disorder are not necessarily confined to the leaves of plants but may occur on any part of the plant structure, including the roots. Recognition of nutrient disorders is but an initial step in any attempt to control or correct a crop abnormality which should be supported by soil and/or plant analyses in conjunction with measurements of crop responses obtained in pot and field studies.

In many developing countries, because of a lack of equipment, chemical reagents and insufficiently trained personnel, to obtain the required analyses is a major problem. Poor infrastructure, a large number of disparate

small farms and transportation problems, complicate the process (BOSTID/NRC, 1982). This, therefore, further underscores the importance of satisfactory and correct recognition and interpretation of symptoms of nutrient disorders in the field in developing countries, as the necessary support services may be inadequate and the results are often too long in coming to be effectively used.

2. CROP ECOLOGY

With the production of food and fiber as his ultimate goal, the farmer considers the response of crops to environmental growth factors. In his efforts to provide a scientific basis for agricultural practices, a scientist aims at the identification of factors limiting crop growth through the measurements of environmental factors. Factors which control plant growth can be considered as:

a. SOIL (Edaphic) FACTORS

(i) root room
(ii) water supply
(iii) air supply
(iv) nutrient supply
(v) harmful (toxic) factors
(vi) soil temperatures

b. ATMOSPHERIC FACTORS

(vii) air temperatures
(viii) air movements
(ix) humidity
(x) light

Many of these factors are closely inter-related, e.g. water supply and air supply are inversely related, water and nutrient supply are directly related and provide reasons for variations in the fertility and productivity.

3. CROP GROWTH RESPONSE TO PLANT NUTRIENTS

As long as a nutritive factor is limiting it limits yield. Nutrient addition in large excess over that needed to maximise yields may depress yields and become harmful (toxic). The present accepted generalized view of the relationship of growth to nutrient supply is a sigmoid relation, where following low initial responses in yield there are rapid yield responses to further nutrient additions until eventually a plateau is reached representing the maximum yield, imposed principally by genetic limitations. The relationship is modified to indicate a possible depressive effective from an excessive nutrient supply.

4. CROP NUTRITIVE ELEMENTS

Generally, crops will absorb any elements which are dissolved in the soil solution. Plants will therefore absorb toxic materials which may be present. Not all of these elements are essential for plant growth. An essential plant nutrient could be described as a nutrient required for growth

and reproduction and no other element may substitute for its function. It is involved directly in the nutrition of plants.

It has been found that about seventeen elements are essential for plant growth (Table 1). Three of these C, H and O come from air and the water absorbed from the soil. Most plants synthesize photosynthate from CO_2 and H_2O using solar energy and H_2O itself is essential for the growth of crops. Six of the other 14 elements, N, P, K, Ca, Mg and S, are also used by plants in relatively large amounts. These are obtained from solids in the soil and are absorbed by plants in large quantities. N, P and S are associated with the organic matter solids in soils. The remaining eight essential nutrient elements Fe, Mn, B, Mo, Cu, Zn, Cl and Co are used by plants in relatively small amounts and are also obtained from solids in the soil. These micronutrients are required in such small amounts that most soils are able to provide them in sufficient quantities for normal plant growth (Brady, 1984).

5. SOIL NUTRIENT ASSOCIATIONS AND NUTRIENT AVAILABILITY

Mineral (inorganic) soils occupy a large proportion of total land area as compared to organic soils with more than 20% (by weight) organic matter. Soil material results from weathering of the earth's crust and the system comprises mineral matter, organic matter (solid phases), water and air (non solid pore phase) which are intimately mixed. This encourages interactions within and between the phases and permits variation in the environment for the growth of plants (Brady, 1984). The top 15cm of a soil (surface horizon) may contain on a volume basis 38% (v/v) mineral, 12% organic matter and 50% pore space. The pore space may contain 15% to 35% water (v/v) depending on the soil's moisture content, with the remaining pore space filled with air. This physical arrangement of the soil components is important when considering the flux of nutrients in the soil system and nutrient release from the solid phase of the soil may result from processes such as ion exchange, decomposition, dissolution or desorption (Barber, 1984).

Because of their small particle size and large specific surface area, particles that comprise the solid phase of soils (for example clays, organic matter and sesquioxides) act somewhat like active chemical substances (soil colloids) and play a significant role in the retention of nutrient ions and/or their release to the roots of plants. Owing to its dependency on soil reaction at some of the exchange sites of these soil particles, a consideration of soil pH is required in any estimation of the capacity of soil to exchange ions.

Additionally, the nature of ions such as Al, Fe, Mn and phosphate, quantities of which are related to or correlated with satisfactory plant growth, depends upon the prevalent pH of the soil/water system. For example both the hydrated Fe (III) ion and the Al ion release H^+ readily,

$$[Al(H_2))_6[^{3+} \rightleftharpoons [Al(OH)(H_2O)_5[^{2+} + H^+ \rightleftharpoons [Al(OH)_2(H_2O)_4]^+ + H^+ \quad \text{(Eq.1)}$$

and maintain soil acidity, whereas phosphate ion exists in equilibrium at

Table 1: Essential nutrient elements and their sources

Used in relatively large amounts		Used in relatively small amounts
Mostly from air and water	**From soil solids**	**From soil solids**
Carbon	Nitrogen	Iron
Hydrogen	Phosphorus	Manganese
Oxygen	Potassium	Boron
	Calcium	Molybdenum
	Magnesium	Copper
	Sulphur	Zinc
		Chlorine
		Cobalt

From Brady, 1984. The Nature and Properties of Soils

the following approximate pH values:

$$\underset{pH=3}{H_3PO_4} \rightleftharpoons \underset{pH=7}{H_2PO_4^- + H^+} \rightleftharpoons \underset{pH=10}{HPO_4^{2-} + 2H^+} \rightleftharpoons PO_4^{3-} + 3H^+ \quad \text{(Eq.2)}$$

In acidic soils therefore Fe^{3+} is more available whereas Ca^{2+} and Mg^{2+} are leached. Phosphate availability may be reduced as iron (III) phosphate and aluminium phosphate is precipitated out of the soil solution where the pH is 5.5 and N may be present as NH_4^+. In soils of high pH values, phosphate availability may be reduced as insoluble calcium phosphate is formed and N may be present as NO_3^-. Microbiological functions are often affected by extremes of pH and loss of activity may be responsible for NH_4^+ accumulation at a pH value below 5.5. An optimum range of pH for overall ion availability is generally accepted between 6.0 and 6.5.

Soil particles have a wide range of types of exchange sites, in addition to permanent and pH dependent sites, which bind ions with different strengths (Barber, 1984) and the relative number of each type varies with the soil. General relationships between associated cations related to the case with which nutritive elements become available to the plant have been developed. These can be used to show nutrient effects that occur when moisture or cation levels change.

6. IMPORTANCE OF NUTRIENT MOBILITY

Ions move from the soil exchange sites through the soil solution to the outer surfaces of plant roots. A nutrient which is not absorbed by soil colloids and remains water soluble may be subjected to leaching losses.

Interactive processes such as diffusion and massflow in porous media have been used to describe the transfer of nutrients through the soil solution to the root surface (Nye and Tinker, 1977).

Evidence from radioisotope studies summarised by Russell (1978) suggests that differential rates of root growth may affect uptake of some nutrient ions differently to others. Ca^{2+} is absorbed near root tips where root hairs are evident but depleted nutrient supply near the root or dried soil ($<$-0.5 MPa) will greatly reduce uptake. Therefore uptake by root systems as a whole may depend disproportionately on younger parts of roots in contact with adequate supplies of water and nutrients.

In soils, the concept of nutrient mobility is quite different to mobility in plants which is primarily concerned with reuse of nutrients at different points within the plant. Diffusion coefficients of plant nutrients are used as an index of ion mobility and in aqueous solutions are of the order of 10^{-5} cm^2 sec^{-1}. In moist soils values for diffusion coefficients are lower and there are large differences between ions (Table 2). In drier soils ion mobility is greatly reduced.

Differences in behaviour between mobile and immobile nutrients are reflected in the volume of their zones of depletion (Table 2). Depletion

zones are correspondingly compressed with diffusion coefficients in drier soils. Therefore roots need to scavenge a larger volume of soil to obtain adequate quantities of nutrients, specifically immobile nutrients.

Tropical soils such as oxisols and ferralsols (lateritic soils) possess more positive charge than temperate soils and there is more anion absorption to soil solids as a result. Thus phosphate may become even less mobile and other anions usually considered mobile, such as nitrate and sulphate may become only moderately mobile. This highlights the importance of rooting density in tropical soils especially when little increase in uptake at rooting densities greater than 2.0cm cm^{-3} was reported for mobile nutrients (Fisher and Dunham, 1984). Whereas for immobile nutrients uptake increased linearly with rooting density (Table 2).

Plants with neighbouring root systems are most likely to be in competition for the mobile nutrients, of which N is the most important. Early establishment of a higher root density may well be the basis of competitive advantage where the soils nitrogen supply is suboptimal.

7. NUTRIENT MOBILITY WITHIN THE PLANT

The ease with which ions pass through the plant varies. Ions containing N, P, K and Mg are more mobile whereas ions containing Ca, S and Fe are among the least mobile (JO/FB Syndicate Bldg, 1981). Therefore, in the detection of ion deficiencies within plants, mobile ion deficiency symptoms will be observed on older leaves first as the ions in short supply move readily to the growing points of the plant where they are in highest demand. The least mobile ions will present deficiency symptoms on newer leaves as they are unable to meet the changing physiological requirements of the young leaves.

Where symptoms of nutrient deficiency appear with equal severity on both old and new leaves the damage may be greater on the older leaves. This usually occurs with elements such as N, P and S which can be conserved as storage materials and can translocate to the younger leaves only if they have not been previously transformed into permanent cell constituents. Although the site of damage is convenient and of value as an aid to recognition, it is not consistently valid, as crops which are able to absorb adequate amounts of the nutritive element in the earlier stages of growth and later are impaired due to a deficiency of the element, will have symptoms of nutrient disorders different to that of a crop in which the nutritive element deficiency occurred throughout the entire growth period.

8. FUNCTIONS OF ESSENTIAL NUTRITIVE ELEMENTS AND CHARACTERISTICS OF NUTRITIVE DISORDERS

8.1 Relatively mobile ion in the plant - symptoms occur on older (lower) leaves

Table 2: The relative mobility of some essential nutrient elements as indicated by the behaviour of their ions

Relative Mobility	In the soil	Diffusion Coefficient $cm^2\ sec^{-1}$	Depletion Zone spread mm	Rooting Densities $cm \cdot cm^{-3}$	In the Plant	Affected Tissue (site)
Mobile	$N\text{-}NO_3^-$	10^{-6}	25	2	N	older
	$S\text{-}SO_4^{2-}$				P	leaves
	$Cl\text{-}Cl^-$				K	grain
					Mg	fruitset
Immobile	$K\text{-}K^+$	10^{-6}	5	8	Ca	younger
	$N\text{-}NH_4^+$	to			S	leaves
	$Mg\text{-}Mg^{2+}$	10^{-7}			Fe	stalk
	$Ca\text{-}Ca^{2+}$				minor	dieback
	$P\text{-}PO_4^{3-}$	10^{-8}	1	$\propto$	nutrients	root system effectiveness

8.1.1 Nitrogen

Nitrogen is a major constituent of all living cells and comprises 40 to 50% of the dry matter of plant protoplasm e.g. proteins, nucleic acids, chlorophyll and coenzymes such as NAD and NADP (Wallace, 1961). It follows that without an adequate supply of N, appreciable growth cannot occur as cell division is inhibited. Thus the shape and size of leaves and reduction of plant growth provides an adverse effect on yield. An increased C/N ratio results in excess carbohydrates and increased cellulose and lignin content. Thickening of cell membranes and increases in lignified tissues result in early maturity. The plant appears small, dry, non succulent with an acute angle of leaf to stem. Leaf colour appears yellowish-green to yellow due to a lack of chlorophyll, and in severe cases brown spots gradually develop to cover an increasing area of leaf, which ultimately dies.

8.1.2 Phosphorus

Phosphorus is closely involved with many physiological plant functions and is principally a constituent of nucleic acid, a major component of the cell nucleus. It is involved in the biochemistry of the metabolism of carbohydrates, proteins and fats through phosphorylated intermediate compounds which act to conserve and provide energy for specific reactions. It stimulates root formation and in particular seed metabolism and germination. Alike N, a shortage of P reduces the rate of cell division stunting the rate of plant growth (vigorous tillering is inhibited). Unlike N shortage, inadequate P provides a high concentration of chlorophyll to the leaves and leaf colour becomes dark green. Plants with red pigments such as carotene in their leaves show a marked red to purple colour at the base of the stem or at the leaf midrib. The leaf colour can change to a dull grey-green and necrosis can set in at the margin finally.

8.1.3 Potassium

Potassium does not enter into the composition of plant constituents such as proteins, chlorophyll, fats, carbohydrates or other leaf pigments which are involved in plant metabolism. It is difficult therefore to precisely define its function but is appears to keep plant cells in a turgid condition by controlling the osmotic pressure of the cell sap and regulates the normal functioning of metabolic processes within the cell. It is involved in enzymatically controlled transphosphorylation reactions and is essential in the synthesis and translocation of carbohydrates, e.g. in pineapples, tomatoes, soyabeans (oil content improved), sugar beet (sugar content improved). It acts as a catalyst in the formation or prevention of the decomposition of chlorophyll. Because of its effects on turgor, K is thought to give rigidity

to stalks and straw and increases the plumpness in grain formation (grain can shrivel if K supplies are inadequate). It improves vigor and disease resistance, hardiness (in legumes), tuber development (root crops) and supplies quality to the crop (tobacco, fruits and vegetables). Its symptoms of nutrient disorders are quite distinctive in the leaf sites as leaf colour changes to an ash-grey, becoming more marked at the tips and leaf margins. The leaves are small, dull and show interveinal chlorosis, so that only tissues along the veins may appear a normal green colour. Necrosis of tissue generally develops at the leaf margins with browning of the tips, but previous to this leaves may pucker and malform with a tendency to curl at the margins, as there is a difference in the distribution of motor cells on the upper and lower leaf surfaces and the osmotic pressure of these cells decreases with inadequate quantities of K. The growth habit of plants tends to be stunted due to shortening of the internodes and shoots may eventually die back at the tips. When the growth of primary shoots is checked, secondary lateral shoots may develop (e.g. extra-tillering in cereals and bushiness in potatoes).

8.1.4 Magnesium

Magnesium, in addition to being a constituent of the chlorophyll molecule performs similar physiological functions to Ca. It appears to be involved in the synthesis of high energy bonds as it is needed for adequate uptake and transfer of P within the plant system. Characteristic changes in leaf colour are obvious to the naked eye with nutrient disorder symptoms due to an inadequate Mg supply. The yellow area is not normally markedly contrasting to the normal leaf colour and generally appears to take the shape of concentric circles. Because of its association with phosphorus and chlorophyll, inadequate supply of Mg may reduce carbohydrate formation (e.g. tubers, fruit juice quality, oil storage).

8.2 Relatively immobile ion in the plant - symptoms occur on young (upper) leaves

8.2.1 Calcium

Calcium has two main functions in plants. Alike K it is involved in regulating the osmotic pressure of the cell sap to maintain turgidity and is a constituent of the middle lamella (chiefly calcium pectate) of the cell wall and other such plant fabrics. It is present in relatively low quantities in seed and fruits. Entry of Ca into the middle lamella is irreversible and if replaced by any other essential element, e.g. K or Mg salts and soluble organic products readily leach through the walls of the cell. Thus Ca is intimately involved in activities at the growing points (meristems) of plants and in root development.

It has been shown to exercise the functions of cell division, cell elongation and detoxification of hydrogen ions (Wallace, 1961). Nutrient disorder symptoms appear in the vigorously growing portions of plants e.g. at the tips of new leaves or at the outer margins and new tissue may fail to develop and appear necrotic.

8.2.2 Sulphur

Sulphur occurs in plants as a constituent of proteins (through amino acids cystine, cysteine and methionine and coenzymes biotin, thiamine), and the sulphur bridges and SH radicles are important structurally and as active chemical sites. It appears to be connected with chlorophyll formation and alike N is involved in parallel roles in plant metabolism. Deficiency symptoms are similar to those of N (reduced chlorophyll content, decreased protein content, increased starch and sucrose but decreased reducing sugars content). However, sulphur of the sulphur proteins of the older leaves is immobile within plants and therefore unlike N, sulphur nutrient disorders are more pronounced in the young tissues.

8.2.3 Micronutrients

Trace elements function primarily as constituents of specific enzymes. Iron is closely concerned with chlorophyll formation and so chlorosis is an outstanding symptom of its nutrient disorder (Table 3). It is a constituent of enzymes concerned with respiration and other oxidation systems (cytochromes b and c, catalase, peroxidase, dehydrogenase, etc.). Its mobility is affected by the presence of Mn, K deficiency, high levels of P and high light intensity. There is evidence of a relationship between quantities of chlorophyll and 'active' (readily soluble) iron in plants (Wallace, 1961). Alike Fe, Mn is also involved with chlorophyll formation and chlorotic patterns arise with Mn deficiencies. It is closely associated with Fe in the plant and elements may show antagonistic effects. Mn in excessive amounts may decrease Fe solubility to cause symptoms of chlorosis and the converse is possible. Cu and Zn are associated with chloroplasts and protein synthesis. Symptoms of nutrient disorders of Cu are highlighted by high protein levels suggesting that proteolysis is impaired. Visual symptoms include blue green leaves and die back effects. A variety of symptoms including little leaf, rosetting of stalks and dieback effects are associated with Zn nutrient disorders. B and Mo are essential anionic nutritive elements. B is involved with inhibition and regulation of many enzymes and has a possible role in the translocation of sugars across membranes. Symptoms of nutrient deficiency of B involve the disorganization of meristems and the breakdown of stalk tissue. Storage organs of plants and fruit are affected. Molybdenum is involved in plant metabolism to reduce NO_3^- to NO_2^-

and is indispensable for normal growth where plants use NO_3-N rather than NH_4-N. Symbiotic bacteria living on the roots of legumes require Mo to fix atmospheric N (Table 3). Symptoms of nutrient disorder such as interveinal chlorosis and mottling appear in new leaves (Ishizuka, 1971).

9. TOXIC FACTORS

Excess of nutrient elements, both essential and non-essential produce toxic effects on plants. With major nutrients there is a fair safety margin for luxury consumption but for the micronutrients the margin could be wide e.g. Mn and Mo, or narrow, e.g. B. As uptake is dependent to a large extent on the presence of the element in its available form in the soil, the ability of an element to have an undesirable effect on growth is inexorably linked to existing conditions in the soil.

Two types of injury may occur:

a. Due to interaction between elements such that an excess of one element (e.g. N or P) may lead to insufficient uptake of K or an excess of K may lead to a reduced uptake of Ca or Mg or an excess of Na may reduce Ca uptake and high concentrations of Ca may reduce K or Fe uptake. These deficiencies will present symptoms of nutrient disorders related to the respective elements.

b. The presence of an element may directly injure the protoplasm or interfere with vital enzyme reactions in plants. For example a high Al ion concentration in acid soils (pH 5.2) in the tropics is a common cause of the failure of agricultural crops. Al ion possibly has two distinct effects:

   (i) a high Al ion concentration in the free space near the root surface may prevent phosphate uptake into the root and

   (ii) Al ion in the living cell may interfere with sugar phosphorylation

10. SOIL CONDITIONS WHERE DEFICIENCY MAY OCCUR

Even when the range between deficiency and toxicity is relatively narrow, deficiency of the element is more likely to occur. A pattern for deficiency could be discerned thus:

a. On sandy soils which are acidic and highly leached, minor as well as major nutrient elements are likely to be deficient as the primary and secondary parent materials may not have contained sufficient of the stable element. Absolute deficiency has occurred.

b. Where some factor (e.g. pH) affects the nutrient element so that it becomes unavailable, a conditional deficiency is present. For example soils with a pH value above 7 are likely to have some minor element deficiency. Except possibly for Mo or Cl, the availability of minor elements is reduced.

Table 3: Micronutrients and secondary nutrients

Micronutrients are absolutely essential for plant growth, and the need for these nutrients varies with crop, soil conditions, and farm management.

For all crops, micronutrients are equally as vital as the primary plant foods. The only difference is the amount - plants need relatively small quantities of micronutrients. Corn, for instance, will contain nitrogen and zinc in the ratio of about 100 to 1. But, the lack of zinc can make the difference between a 100-bushel per acre harvest and a crop failure. This "Hidden Hunger" for one or more micronutrients or secondary nutrients can be very costly in the long run.

Constant attention should be given to micronutrient levels and needs. A crop can suffer from "Hidden Hunger" without showing a specific deficiency symptom. If the symptom is evident, the crop is already suffering a yield reduction. It would be an economic safeguard to check-and avoid-or, at least, check-and-remedy any yield-robbing "Hidden Hunger" problems. The following table is a guide to the factors which affect micronutrient and secondary nutrient deficiencies - and the crops most susceptible to these deficiencies.

Micronutrients and secondary nutrients.

Micronutrient or secondary nutrient	Functions within the plant	Soil type and conditions where deficiency may occur	Crops most susceptible to this nutrient deficiency
Boron (B)	Essential to actively growing tissue in the new growth; necessary for pollen viability and good seed set.	Occurs in acid-leached soils, coarse-textured sandy soils, peats and mucks, drought conditions, over-limed soils, alkaline or low-organic matter soils.	beets, citrus, cotton, cauliflower, cabbage, celery, corn, sweet potatoes, tomatoes, tree crops, and tobacco
Copper (Cu)	A major part of a necessary photo-synthesis enzyme. Very important during the plant's reproductive stage.	Occurs in sandy soils, peats and mucks, over-limed soils, and in high concentrations of iron and manganese.	Small grains, corn, vegetables, tree fruits carrots, onions.
Iron (Fe)	Promotes formation of chlorophyll.	Occurs in alkaline soils; in calcareous soils when cold and wet; insoils where phosphate has been excessively applied	Beans, soybeans, corn, sorghum, tree fruits, ornamentals, grasses, milo, lemons, limes and rice.

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Manganese (Mn)	A part of important enzymes involved in respiration and protein synthesis.	Occurs in sands, peats and mucks, alkaline (pH of 6.5 or above) and particularly in calcareous over-limed soils. Also, areas with low organic matter.	Soybeans, small grains, tree fruits, cotton, leafy vegetables, dry beans
Molybdenum (Mo)	Transforms inorganic nitrogen to organic. Essential for nitrogen fixation by nodule bacteria in legumes.	Occurs in acid soils (low pH) and in highly-weathered acid-leached soils. And in soils with low phosphate levels.	Cauliflower, citrus, all legumes.
Zinc (Zn)	A growth regulator. Important as a catalyst and regulator in plant's use of other nutrients.	Occurs in calcareous soils (pH of 6.0 or above) after leaching and erosion; in acid-leached soils; in coarse sands; and in soils where phosphate has been excessively applied. Also in low-organic matter, or over - limed soils.	Beans, soybeans, citrus corn, sorghum, onions, potatoes, tree fruits, cotton, milo, rice, sweet corn.
Calcium (Ca)	A secondary nutrient found in greatest amount in leaves. Utilized for continuous cell division and involved in nitrogen metabolism. Corrects soil acidity and improves structure.	Occurs in low pH soils. Also in areas of high nitrogen applications, and high potassium levels. Particularly prevalent in the upland soils of the old cotton belt.	Vegetable crops, tree fruits, cotton, potatoes, tomatoes, celery, citrus, soybeans, most other legumes.
Magnesium (Mg)	A secondary nutrient. Participates in the activity of enzymes. Assists in translocation of phosphorus in the plant. Found mostly in the chlorophyll-bearing tissues of a plant.	Occurs in low pH soils. Also in soils where excessive potash has been applied, or in areas of high calcium-lime use.	Cotton, cabbage, carrots, celery, cron cucumbers, melons, squash, snapbeans, tree fruits, small grains, onions, potatoes, tobacco, sugarcane, tomatoes, turnips, corn, and citrus.
Sulfur (S)	A secondary nutrient necessary for the formulation of cystine - an amino-acid that forms protein.	In areas of low soil sulfate levels, and where there is excessively available nitrogen on low organic matter soils. In areas where there has been inadequate sulfate fertilization. (There should be one pound of sulfur for every ten pounds of available nitrogen).	Citrus, corn, sorghum, cotton, small grains, legumes, sugar cane, tomatoes, potatoes, and many other vegetables.

Table 4: Micronutrient availability

The main objective of any farming program is to maximize crop yield with the inputs and resources available. In order to accomplish this objective, the crop's fertility program must be in balance. When a major or secondary nutrient tests high in the soil, the addition of a micronutrient or another secondary nutrient may be necessary to offset plant uptake interference or nutrient imbalance.

Generally, cold, wet soils tend to aggravate a micronutrient deficiency. Alkaline or high pH soils tend to tie up micronutrients as do high levels of phosphate fertilizer. Many things affect availability. The following chart pinpoints the major factors.

Factors which affect micronutrient and secondary nutrient availability

Deficiency observed:									Cause of deficiency:
S	Ca	Mg	Mn	Fe	B	Cu	Zn	Mo	(Soil nutrient imbalances)
•	•	•		•		•			high nitrogen
		•		•		•	•		high phosphorus
				•					low potassium
	•								low calcium
		•		•	•				high calcium
									high magnesium
				•		•		•	high manganese
			•			•			high iron
			•	•				•	high copper
					•				low zinc
			•	•	•				high zinc
	•	•	•					•	low pH
		•	•	•	•	•	•		high pH
								•	high sulfur
	•	•	•						high sodium
				•					high bicarbonates
				•					iron:copper;manganese imbalance
									(Other soil conditions)
•				•	•	•			low organic matter
			•			•	•		high organic matter
		•	•						poor drainage
			•		•				drought
	•	•	•	•					cold, wet soils
	•	•		•					poorly aerated soils
•	•	•					•		exposed subsoils
			•						heavey manuring
			•	•	•	•			heavy rainfall
•	•	•	•		•	•	•		light and sandy soil

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c. In organic soils micronutrient deficiency is likely to be prevalent as the availability of these nutrient elements is affected by the formation of inorganic - organic complexes with the humic colloids.

d. Where crops are intensively grown on heavily fertilized mineral soils, the soil supply of the micronutrients may be too heavily taxed and the requirement for minor elements becomes definitive.

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