Guidelines for surveillance for plant pests in Asia and the Pacific

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Australian Government

Australian Centre for International Agricultural Research

Rural Industries Research and Development Corporation

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Foreword

Countries negotiating trade in agricultural commodities that may provide pathways for moving pests into new areas must be able to access information on the biology, distribution, host range and economic status of plant pests.

While plant health has become a trade policy issue, knowledge of the health status of a country's agricultural and forestry industries has other important applications. These include the development of robust quarantine policies and the management of endemic pests.

Plant health problems affect society in many ways. As yields are reduced, farmers' incomes are similarly affected. Consumers have less food and fewer food choices or the food may contain chemical residues. As well, many areas of society may be affected by incursion of new pests, diseases and weeds into a community.

Virtually all of Australia's livestock and cropping industries are based on exotic germplasm. Through rigorous quarantine action over the last 100 years Australia is free from many serious exotic pests and diseases. The favourable health status of Australia's agricultural industries provides a competitive advantage in accessing foreign markets.

It is important to all of ACIAR's partner countries to know what plant and animal health problems occur in their territories. ACIAR has previously published instruction guides on how to survey for animal health problems and aquaculture health problems. ACIAR has also helped individual developing countries to survey specific pests — for example, fruit flies in a number of Asian and South Pacific countries, whiteflies in the South Pacific and others. However, no systematic attempt has been made to give countries generic skills to undertake their own surveys in the field of plant health.

Production of this manual has also been supported by the Rural Industries Research and Development Corporation (RIRDC). It is important to RIRDC that Australia has the capacity to take pre-emptive actions to mitigate threats posed by exotic pests. This manual, through training plant health authorities in regional countries to describe the health status of their crops, allows Australia to address pest threats at source rather than after they are detected in Australia.

This manual will assist plant health scientists to devise surveillance programs and to transmit specimens to the laboratory for identification and preservation. Countries can then begin to share results of the surveys with each other and this should lead to increasing wider cooperation in plant health research.

This publication is available for free download from ACIAR's website www.aciar.gov.au.

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Preface

In 2001–02, the Australian Agency for International Development (AusAID) funded the Office of the Chief Plant Protection Officer, Australian Government Department of Agriculture Fisheries and Forestry (DAFF) to report on the state of the arthropod pest collections and plant disease herbaria in the ASEAN countries. The work was undertaken in collaboration with ASEANET.[1] In their reports[2], the authors concluded that none of the countries of the region had a capacity to provide an adequate description of the health status of its crops. The problem was attributed, in large part, to the small numbers of specimens of plant diseases held in biological collections. The arthropod pest collections were generally much better populated than the plant disease herbaria, but all would benefit from additional resources and assistance to bring them up to contemporary international standards.

Pest[3] collections are significant because they provide the most reliable evidence of the plant health status of a country. These records are the foundation for developing robust policies for domestic and international quarantine and for developing pest-management strategies at the farm level. The collections have taken on particular significance since the establishment of the World Trade Organization (WTO) in 1995, which was heralded as opening a new era in trade liberalisation.

Unlike its predecessor, the General Agreement on Tariffs and Trade, the WTO is a rules-based organisation, with the rules governing trade in agricultural commodities set out in the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement). While trade in agricultural commodities has expanded since 1995, exports from developing countries have not expanded to the same extent as trade between the developed members. The developed countries have expanded exports by using the rules of the SPS Agreement to prise open markets previously closed on questionable quarantine

1 ASEANET is the South East Asian LOOP (Locally Organised and Operated Partnership) of BioNET INTERNATIONAL, a body that works collaboratively to develop regional self sufficiency in taxonomy and biosystematics.

2 Evans, G., Lum Keng-yeang and Murdoch, L. 2002. Needs assessment in taxonomy and biosystematics for plant pathogenic organisms in countries of South East Asia. Office of the Chief Plant Protection Officer, Department of Agriculture, Fisheries and Forestry, unpublished report.

Naumann, I.D. and Md Jusoh, M. [Md Jusoh Mamat] (2002). Needs assessment in taxonomy of arthropod pests of plants in countries of South East Asia: biosystematics, collection and information management. Office of the Chief Plant Protection Officer, Department of Agriculture, Fisheries and Forestry, unpublished report.

3 The term is used herein to include arthropod pests and plant pathogens.

grounds. At the same time, governments in the many countries are under pressure from their farmers to use the rules to exclude commodities that they see as posing a threat to their industries. Plant health has become a major trade-policy issue.

A country that cannot provide an adequate description of the health (pest) status of its agricultural industries is at a disadvantage when negotiating access to foreign markets. Prospective importers will assess risk based on their knowledge of the pests in the country seeking to export, the likelihood of introducing exotic pests of concern with the imported commodity and the availability of phytosanitary measures to reduce risk to an acceptable level. Extensive specimen-based records are the key for developing countries to negotiate with developed countries on a fair trading system.

Many collections of arthropod pests and plant diseases are the product of work dating back a century or more. The early curators of these collections sourced specimens from practising plant-health scientists, farmers and from their own collecting trips. While specimens submitted by plant-health scientists and farmers are still valuable, the collection of specimens has become more purposeful than in the past, driven by the need to expand scientific knowledge about biodiversity, concern about the need to recognise alien pests in new environments and a desire to expand trade in agricultural commodities.

Countries wanting to expand exports of agricultural commodities under the rules of the WTO do not have the luxury of building their pest collections over an extended period. Nor do they have to. The development of specimen-based pest lists can be accelerated through structured surveillance programs, focusing on the pests that might be carried on the commodity to be exported. Often the trading partner will specify the extent of the surveillance activities to be undertaken, but not always. These guidelines have been written with a view to helping plant-health scientists needing to undertake surveillance activities, for whatever purpose.

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Glossary[4]

area

An officially defined country, part of a country or all or parts of several countries

area of low pest prevalence

An area, whether all of a country, part of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific pest occurs at low levels and which is subject to effective surveillance, control or eradication measures

delimiting survey

Survey conducted to establish the boundaries of an area considered to be infested by or free from a pest

detection survey

Survey conducted in an area to determine if pests are present

general surveillance

A process whereby information on particular pests which are of concern for an area is gathered from many sources, wherever it is available and provided for use by the NPPO

International Plant Protection Convention (IPPC)

An international convention deposited with FAO in Rome in 1951 and as subsequently amended

International Standard for Phytosanitary Measures (ISPM)

An international standard adopted by the Conference of FAO, the Interim Commission on Phytosanitary Measures or the Commission on Phytosanitary Measures, established under the IPPC

international standards

International standards established in accordance with Article X paragraph 1 and 2 of the IPPC

[4] For International Standards (ISPMs) and definitions, see: International Phytosanitary Portal at <https://www.ippc.int/IPP/En/default.jsp>, the official website of the International Plant Protection Convention.

monitoring survey

Ongoing survey to verify the characteristics of a pest population

National Plant Protection Organization (NPPO)

Official service established by a government to discharge the functions specified by the IPPC

> The IPPC (1997), in relation to its main purpose of "*securing common and effective action to prevent the spread and introduction of pests of plants and plant products,* (Article I.1) requires countries *to make provision, to the best of their ability, for an official national plant protection organization,*" (Article IV.1) whose responsibilities include the following:
>
> "*…the surveillance of growing plants, including both areas under cultivation (*inter alia *fields, plantations, nurseries, gardens, greenhouses and laboratories) and wild flora, and of plants and plant products in storage or in transportation, particularly with the object of reporting the occurrence, outbreak and spread of pests, and of controlling those pests, including the reporting referred to under Article VIII paragraph 1(a)…*" (Article IV.2b).
>
> **ISPM 17**

non-quarantine pest

Pest that is not a quarantine pest for an area

pest

Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products

pest free area (PFA)

An area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained

pest free place of production (PFPP)

Place of production in which a specific pest does not occur, as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period

pest free production site (PFPS)

A defined portion of a place of production in which a specific pest does not occur, as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period and that is managed as a separate unit in the same way as a pest free place of production

pest record

A document providing information concerning the presence or absence of a specific pest at a particular location at a certain time, within an area (usually a country) under described circumstances

pest risk analysis (PRA)

The process of evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it

pest status (in an area)

Presence or absence, at the present time, of a pest in an area, including, where appropriate, its distribution, as officially determined using expert judgment on the basis of current and historical pest records and other information

quarantine pest

A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled

Regional Plant Protection Organization (RPPO)

An intergovernmental organisation with the functions laid down by Article IX of the IPPC

regulated pest

A quarantine pest or a regulated non-quarantine pest

regulated non-quarantine pest (RNQP)

A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party

specific surveys

Procedures by which NPPOs obtain information on pests of concern on specific sites in an area over a defined period of time

surveillance

An official process which collects and records data on pest occurrence or absence by survey, monitoring or other procedures

survey

An official procedure conducted over a defined period to determine the characteristics of a pest population or to determine which species occur in an area

Abbreviations

ALPP	area of low pest prevalence
APHIS	Animal and Plant Heath Inspection Service
APPPC	Asia Pacific Plant Protection Commission
AQIS	Australian Quarantine and Inspection Service
ASEAN	Association of Southeast Asian Nations
ASEANET	South East Asian LOOP of the BioNET INTERNATIONAL
AusAID	Australian Agency for International Development
EPPO	European and Mediterranean Plant Protection Organization
FAO	Food and Agriculture Organization of the United Nations
GPS	geographical positioning system
ICPM	Interim Commission on Phytosanitary Measures
IPPC	International Plant Protection Convention
ISPM	International Standard for Phytosanitary Measures
ISSG	Invasive Species Specialist Group
LOOP	Locally Organised and Operated Partnership
NAPPO	North American Plant Protection Organization
NAQS	Northern Australia Quarantine Strategy
NPPO	National Plant Protection Organisation
PFA	pest free area
PFPP	pest free place of production
PFPS	pest free production site
PNG	Papua New Guinea
PRA	pest risk assessment
QDPI&F	Queensland Department of Primary Industries and Fisheries
RPPO	Regional Plant Protection Organization
RSPM	Regional Standard for Phytosanitary Measures
SPC	Secretariat of the Pacific Community
SPS	Sanitary and Phytosanitary Measures
USDA	United States Department of Agriculture
WTO	World Trade Organization

Chapter 1

How to use these guidelines

1.1. Scope and readership

These guidelines were written to assist plant-health scientists design surveillance programs for detecting arthropod pests and plant pathogens in crops, plantation forests and natural ecosystems. The publication covers the planning of surveillance programs for building specimen-based lists of pests[5], surveillance for monitoring the status of particular pests, surveillance for determining the limits of distribution of pests, surveillance for determining the presence or absence of pests in particular areas, and general surveillance.

Those who were initially responsible for planning the production of these guidelines had in mind the needs of plant-health scientists in developing countries of the region, particularly those countries wanting to build specimen-based pest lists to support negotiations to expand trade in agricultural commodities. To that end, the Australian Centre for International Agricultural Research (ACIAR) and the Rural Industries Research and Development Corporation (RIRDC) provided sufficient funds to involve plant-health scientists from a number of the developing countries in Southeast Asia and the Pacific in the production of these guidelines. ACIAR also provided funds to engage selected specialists from Australia in the process. Together, the regional and Australian specialists constituted a 'reference group' that convened in Canberra, Australia, in November 2004 to oversee the production of this publication. The reference group was concerned that the manual should not be too prescriptive, noting that the approach to surveillance for plant pests needs to be flexible, taking into account such matters as the resources available and difficulties in accessing some sites where pests might be found. With these limitations in mind, the reference group was of the view that the word 'guidelines' should appear in the title rather than calling the publication a 'manual' or 'toolbox'. A number of the members of the reference group also volunteered contributions that form the case studies at the end of the guidelines, based on surveys for plant pests in selected countries of Southeast Asia, some Pacific island countries and Australia.

5 The term pest is used throughout this publication in a generic sense and includes reference to arthropods, plant pathogens and weeds.

The guidelines take the reader through a series of easy-to-follow steps to design a surveillance program, emphasising the need to carefully document the process. At each step, useful tips are provided on things to think about in advancing a surveillance plan. The guidelines also provide advice on how to approach the critical issues of how to design a statistically valid surveillance program that will meet the most rigorous demands of bureaucrats, trading partners and others who must have faith in the results, for whatever purpose the surveillance is undertaken.

The reference group, ACIAR and those who were responsible for the production of this publication expect that it could be used by any plant-health scientist planning a surveillance program. Those scientists who are novices at surveillance should find the guidelines particularly useful. The process of planning a surveillance activity drawing on these guidelines will quickly build the confidence of any novice and greatly improve the design of pest surveillance programs.

1.2. ISPMs and terminology used in these guidelines

International standards have been developed to guide how trade in agricultural commodities can be achieved with the lowest possible risk of moving pests between the trading countries. The main standards are the series of International Standards for Phytosanitary Measures (ISPMs). These have been developed and endorsed by the Interim Commission on Phytosanitary Measures (ICPM) under the aegis of the International Plant Protection Convention (IPPC). The purpose of the IPPC is to secure common and effective action to prevent the spread and introduction of pests and to promote measures for their control. Contracting parties to the IPPC have the right to use phytosanitary measures to regulate the entry of articles, including whole plants and plant products, capable of harbouring plant pests.

As international standards have been developed that relate to surveillance for plant pests, the guidelines in this book have included and followed the ISPMs whenever possible. As the standards were written to encompass many countries and situations, it has been necessary in these guidelines to provide a great deal more information about designing surveys than is in the standards. Wherever the ISPMs are relevant to sections in the guidelines, the appropriate ISPM passages are given. It should be noted that the ISPMs primarily target trade-related surveillance, which is not the only reason surveillance is performed. These guidelines cover the design of surveys for most purposes, including trade-related activities.

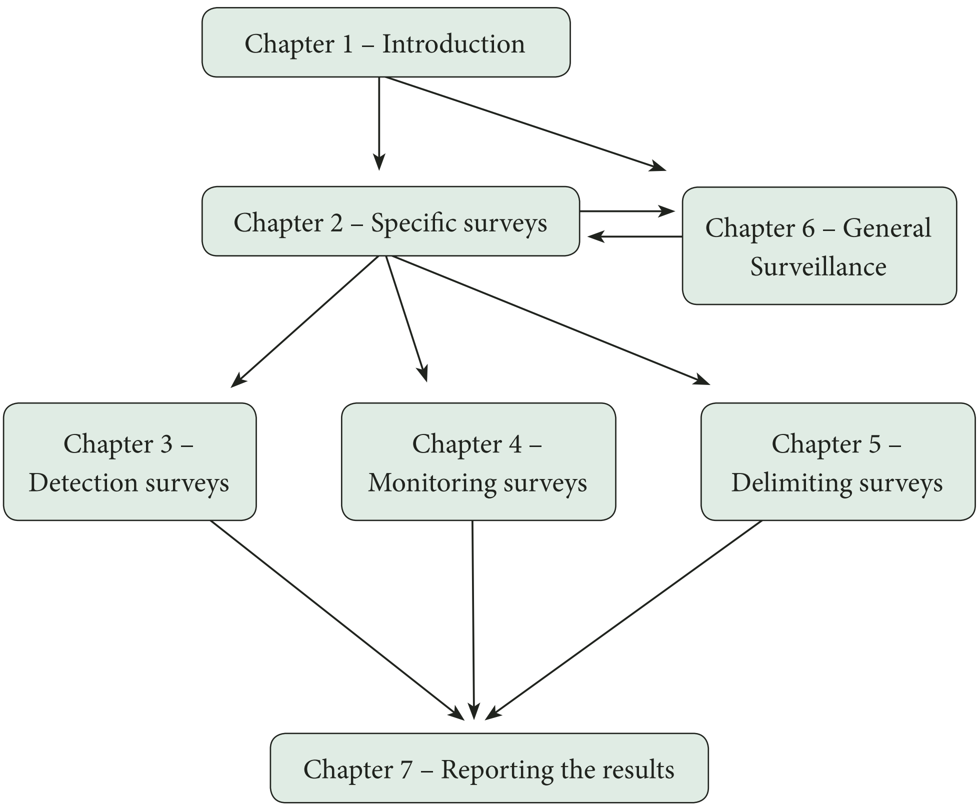
Whenever possible, ISPM definitions are used in the guidelines. The glossary of ISPM terms that relate to surveillance is published in ISPMs 5 and 6. The most relevant entries are reproduced in the glossary of these guidelines.

An important distinction to be made at the outset is use of the terms 'general surveillance' and 'specific surveys'. Often, people misunderstand 'general surveillance' to mean performing a field survey for all kinds of (general) pests. On the contrary, general surveillance is an umbrella term that is not clearly defined in the ISPMs. In these guidelines, the term is understood to include a range of activities. The first and most common use is the gathering of information about a particular pest. Other activities include public-awareness campaigns as well as reporting networks specifically for NPPOs. Specific surveys are those survey activities that involve field work; so specific surveys include surveys that look 'generally' for pests or for 'general' pests in the field.

1.3. How best to use these guidelines

The focus of these guidelines is to provide guidance on how to design specific surveys. The ISPMs divide specific surveys into three categories: detection surveys, monitoring surveys and delimiting surveys. Chapter 2 is the most important chapter of these guidelines and should be read and understood, irrespective of what type of survey you intend to design. Chapter 2 provides information about the basic components and content for any specific survey. The design is set out in 21 steps. The first 20 steps are in Chapter 2. Step 21—Reporting the results—is covered in Chapter 7.

Chapters 3, 4 and 5 provide additional information about the three ISPM categories of specific surveys and each relates back to Chapter 2. Chapter 6 is dedicated to general surveillance. Chapter 7 details how to report survey findings. Chapter 8 includes a number of examples of specific surveys that cover a wide range of pests and conditions. These case studies were contributed by numerous plant-health experts from the Southeast Asian and Pacific regions and Australia.



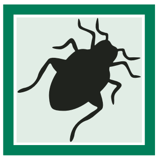
1.4. Symbols in the text

Symbols have been added throughout the text to draw the attention of people who are particularly interested in one or other of four main topics: weeds, forests, plant pathogens, and insects and allied forms. The key to the symbols is:

Forests

Weeds

Plant pathology



Insects and allied forms

Chapter 2

Designing a specific survey

2.1. Introduction

Specific surveys involve field work—going out and looking for the pests. This chapter covers the steps on how to decide where to look, how many places to look in and what sort of data to collect. The chapter goes on to provide information on how to collect and preserve specimens, followed by discussion of other important considerations to make the most of your survey, including guidance on what to do with the data collected.

Before you can go into the field and begin looking for pests, there are many planning decisions to be made. A survey plan needs to be robust, and the results should represent the actual pest status. The plan needs to be feasible both physically and financially.

There are no hard and fast rules about the correct number of samples, or one correct way of designing a survey. Because of this, it is important that the reasons for the design steps chosen are transparent.

When planning a new survey, the details of the design need to be carefully recorded and justified. If you provide justifications, or reasons for particular choices, it will be easier and faster for you or someone else to design similar survey plans. By providing reasons, you will also assist anyone who might later use your report as part of general surveillance. Your reasons and decisions may need to be justified if the plan requires approval from an NPPO.

While some decisions may change when the plan is put into practice, these changes can be added along with reasons for the changes.

The remainder of this chapter describes the 21 steps involved in the design and conduct of a survey. These are shown in Figure 1.

2.2. Step 1. Choosing a title and recording authors

Choose a simple title for your plan. You may wish to revise it as you go along.

Include the names of the people responsible for producing the survey plan and where they can be contacted.

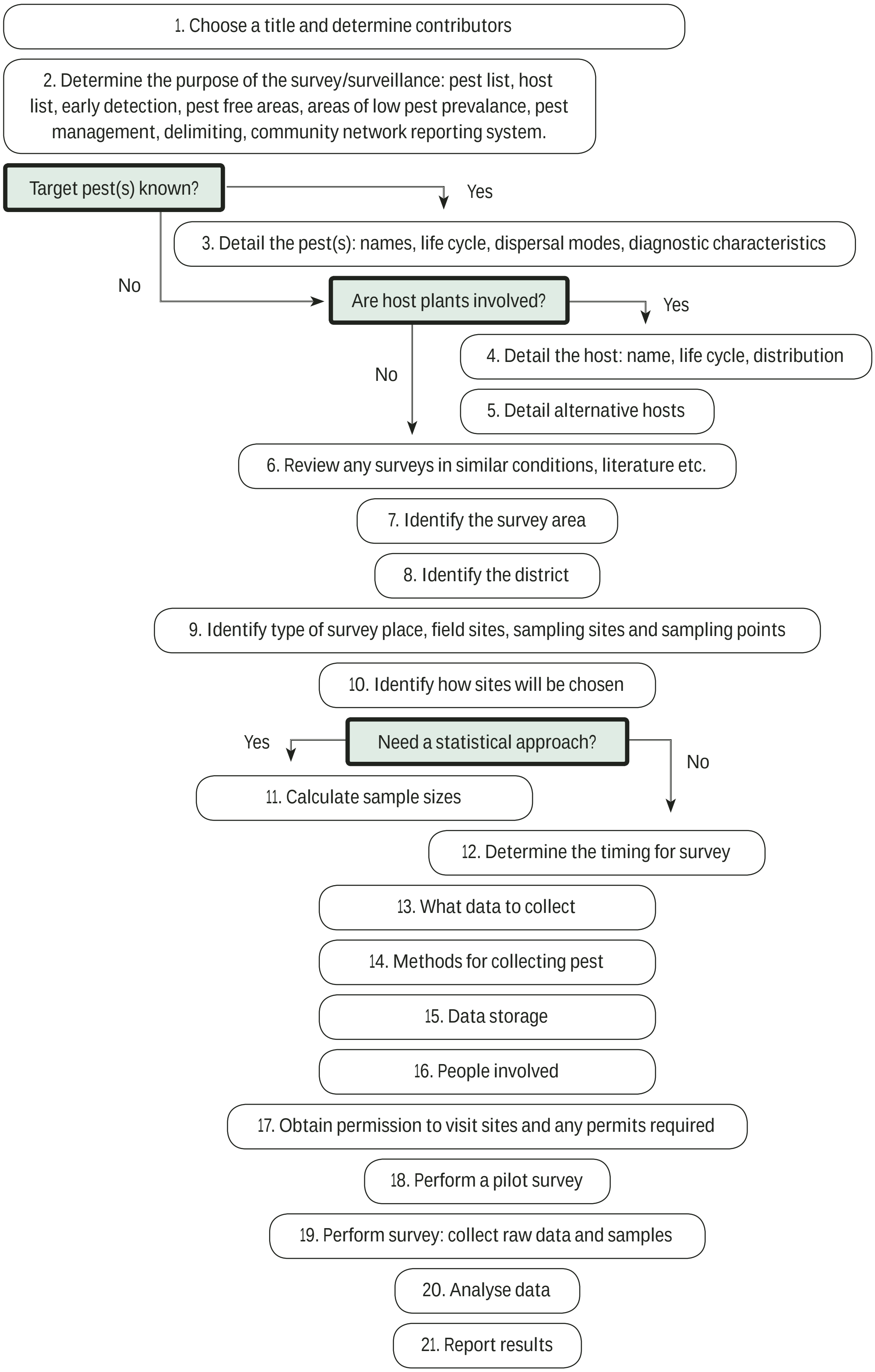


Figure 1. Steps to designing a specific survey.

Step 1

- ▶ Record the title of your survey.
- ▶ Record the names of authors.

2.3. Step 2. Reasons for surveying

There are many reasons for surveying pests. As discussed in Chapter 1, some of the reasons are:

- to develop a list of pests or hosts present in an area
- to demonstrate a pest-free area (the absence of a particular pest in an area) or places of low pest prevalence for trade purposes
- to develop a baseline list of pests before ongoing monitoring for changes in pest status
- for pest management and control
- for early detection of exotic pests
- for early detection of established organisms becoming pests
- to delimit the full extent of a pest following an incursion
- to monitor progress in a pest eradication campaign.

You may have other reasons that are combinations of the above.

Box 1. Surveying to test an association

If you are trying to see if the presence of a pest is associated with another factor, such as a particular type of place (for example, on road verges or near mobile-phone towers) or variety of host, then an experiment testing the hypothesis needs to be designed. This 'hypothesis testing' is different from surveillance.

Testing an association must be very carefully designed to exclude all other possible explanations of pest distribution and be able to isolate the factor. In this situation, you would need to test if the effect was true or false without biasing the results. Such experimental design is not covered in these guidelines. For more information, search for the term 'hypothesis testing' on the Internet.

Step 2

- ▶ Record the purpose of your survey.

2.4. Step 3. Identify target pests

If the targeted pests are not yet known—for example, you intend to survey for new weeds—skip ahead to Step 4.

If you do know which pests you intend to survey, this step involves gathering as much information as possible about the pests.

2.4.1. Useful sources of information

Finding information on pests—their life cycles and identifiable characteristics—can be easier for pests that are already present in a country, because there are likely to be local and overseas experts (entomologists, pathologists, plant health and quarantine officers). Information on exotic pests can be obtained from countries where the pest is known to be present. This may involve contacting the agricultural department of the government (in particular, the NPPO), by finding published material or by searching on the Internet (be careful to assess the credibility of the source of the information). There are numerous lists and databases that can be accessed that describe a wide range of pests, e.g. the CABI Crop Protection Compendium.

From ISPM 6 (FAO 1997, p.7):

> These [information] sources may include: NPPOs, other national and local government agencies, research institutions, universities, scientific societies (including amateur specialists), producers, consultants, museums, the general public, scientific and trade journals, unpublished data and contemporary observations. In addition, the NPPO may obtain information from international sources such as FAO, Regional Plant Protection Organizations (RPPOs), etc.

Other sources could be:

- existing PRA reports, either conducted by your own country or by agencies of other countries
- reference collections of insect pests and plant pathogens of agricultural importance
- pest and disease interception databases from quarantine authorities
- the Internet (see Box 2, page 24).

2.4.2. Verifying the information sources

ISPM 8 has a basis for evaluating the reliability of a pest record that could equally be applied to assessing information sources to be used in developing your survey. The relevant elements in a table provided in ISPM 8 are the categories of expertise of contributors and the quality of written information sources. Examine any available sources of information in terms of authoritativeness of the people associated with the material and the quality of the information provided.

2.4.3. Pest names

Begin by creating a list of the scientific and common names of the targeted pests. Include synonyms.

2.4.4. Pest vectors

Identify any vectors of the pests that are to be surveyed. If the pests have vectors, they will need to be included in your list of target organisms.

2.4.5. Possible pest impacts

Consider why these pests are chosen—are they regarded as major pests or pest threats? Do trade partners want more information on the status of specific pests in your area?

In general terms, describe how the pests would be likely to affect a host, production system or ecosystem, and the industry as a whole.

2.4.6. Pest characteristics: how would the pest be identified in the field?

The diagnostic characteristics of a pest, or symptoms of its presence, can be compiled from many sources. For pests that are already present in a country, farmers and foresters may be familiar with the pest. Ensuring that the pest has been correctly identified may require confirmation by a plant pathologist for plant pathogens, an entomologist for insects and allied forms, or a botanist for weeds. You may need to create a list of specialists and laboratories that have experience with the pests and the diagnostic capacity to identify them, depending on what pests you intend to survey.

Where host plants are involved, describe the parts of the plants most likely to be infested or infected, and which parts of the plant should be examined, e.g. stem, bark, leaves, roots, crown, base of plant. Does the pest target a commodity, e.g. fruit or grain? Is the pest associated with particular stages of a host plant's growth? Is the pest attracted by light or pheromones? Describe where the pest or the characteristic symptoms would be found on the host or commodity; for example, flying above a crop, bored into bark, the underside of leaves, frass at the base of plant, presence of curly leaves, growing along the crop rows. A botanist can assist in identifying the range of possible hosts for a plant pest. Are there any factors that affect symptom development, such as host cultivar, growth stage, season, pesticide application and climatic conditions?

Include all available information about the pest's life cycle.

2.4.7. Collecting reference specimens and images

For both general and specific surveys, images of the diagnostic features of the pest and any effects on host plants would be useful for reports. Having handout material that can be used in the field may be critical to detection, particularly if the pest has not been seen before by the surveillance team. Having a reference collection of pressed examples of plants or affected plants, or small specimen collections of invertebrate animals may also prove useful as long as they are not cumbersome and can be protected from damage. Electronic images can be collected from a number of sites on the Web, photographed using a digital camera, or you may request them from colleagues or email networks. These can be used to create pest information sheets.

Box 2. Internet resources for pest information

Animal and Plant Heath Inspection Service (APHIS) of the USDA

At: <http//:www.aphis.usda.gov/ppq/index.html>

This website has links to the North American Plant Protection Organization (NAPPO) standards as well as the International Standards for Phytosanitary Measures. The site has manuals on a number of invertebrate pest species, with useful information on identification, survey methods and pest control. Pest risk assessments of commodities being considered for import into the United States are available for numerous pests and these can provide readily accessible information about host ranges and surveillance methods, amongst other useful sections. APHIS also provides a useful website at <http//:www.invasivespecies.gov/databases> with links to a wide range of pest information databases; for example, those databases listed in this box under HEAR and ISSG, journal article databases and some dealing with aquatic pests.

American Phytopathological Society (APS)

At: <www.apsnet.org>

APSNet contains discussions of plant pathogens through newsletters, and a limited image collection. It also contains a database of pest lists for different crops and commodities (see ‘Common names of plant diseases’ under ‘Online resources’ and type in a host or pest name). The Society produces four journals available on subscription: Phytopathology, Plant Disease, Molecular Plant–Microbe Interactions and Plant Health Progress.

CAB International (CABI)

At: <www.cabi.org>

CABI aims to generate, disseminate and encourage use of knowledge in the applied biosciences field. This includes the areas of human welfare and the environment. CAB International publishes numerous books and other reference material that are listed online at <www.cabi-publishing.org>. CABI publishes a comprehensive database of abstracts from scientific publications. This is available via subscription on CD and online.

CABI Crop Protection Compendium

The compendium contains fact sheets on a wide diversity of pests. To use the compendium online or from CD, a licence must be purchased and the software installed on a computer. More information and a free trial are available at <www.cabicompendium.org/cpc>.

Diagnostic Protocols (DIAGPRO)

At: <www.csl.gov.uk/science/organ/ph/diagpro>

This website is coordinated by the UK Central Science Laboratory to produce diagnostic protocols for fifteen organisms that are harmful to plants.

These protocols provide information about sampling, in addition to diagnostic features and methods.

European and Mediterranean Plant Protection Organization (EPPO)

At: <www.eppo.org>

This organisation coordinates numerous aspects of plant protection across most of the European countries. EPPO has produced a number of standards on phytosanitary measures and plant protection products. While these standards need apply only to dealings with the European Community, they also provide insight into the quarantine barriers in use. Some of the standards provide a list of pests and information about their control for different crops and about identification in the field (see ‘Good plant protection practice’ and ‘Phytosanitary procedures’ under ‘Standards’).

Germplasm Resources Information Network (GRIN)

At: <www.ars-grin.gov/cgi-bin/npgs/html/index.pl>

This site can provide information about taxonomy of plants. It permits searches at family, genus and species levels, as well as for common names. While it is not clear how to navigate the site (currently), it is worth persevering as the database is extensive.

Global Invasive Species Programme (GISP)

At: <www.gisp.org>

This program is partnered by the Convention on Biological Diversity. The GISP website largely discusses invasive species in general terms and provides useful links, such as those in this box. The CBD website (<www.biodiv.org/programmes/cross-cutting/alien>) has a number of case studies on a diverse range of invasive species, including those affecting agriculture.

Hawaiian Ecosystems At Risk (HEAR)

At: <www.hear.org>

The Hawaiian Ecosystems at Risk project aims to provide information and resources to assist in management of exotic invasive species in Hawaii and the Pacific.

The website contains links to a global compendium of weeds at <www.hear.org/gcw>. This compendium has unillustrated fact sheets containing what limited information has been collected to date. The sheets cover alternative names, pest status, origin, environmental extremes tolerated and whether or not the plants are cultivated.

The HEAR website contains links to the report: ‘Invasive species in the Pacific. Technical review of regional strategy’, produced by the South Pacific Regional Environment Programme (SPREP). This report reviewed the pests that posed threats to the Pacific region when written in 2000. See <www.hear.org/AlienSpeciesInHawaii/articles>.

International Plant Protection Convention (IPPC)

At: <www.ippc.int/IPP/En/default.htm>

The IPPC website contains the ISPM standards and links to other multinational plant protection organisations.

Invasive Species Specialist Group (ISSG)

At: <www.issg.org>

This site has two useful products: a list-server of specialists; and the Global Invasive Species Database.

ALIENS-L is an email list-server of the Invasive Species Specialist Group (ISSG) of the World Conservation Union (IUCN) Species Survival Commission, organised through the SPC. This is a discussion forum for any type of invasive organism and so the topics can be broad. This is an easy way to ask questions of an expert group.

To subscribe to the email list send an email to <Aliens-L-request@indaba.iucn.org>, with a blank subject line, and 'join' in the text field.

The Global Invasive Species Database provides information on species that threaten biodiversity, and covers both plants and animals.

The database can be found at <http//: www.issg.org/database/welcome/>.

Landcare Research, New Zealand

At: <www.landcareresearch.co.nz/databases/index.asp>

Landcare Research holds a number of biological and resource collections and databases. Lists of specimens held in collections are provided, which may be a useful resource if you require specimen copies, assistance with diagnosis or are looking for useful electronic images of pests. The collections include nematodes, arthropods, fungi and other pathogens, and plants that are native to New Zealand.

Pacific Island Ecosystems at Risk (PIER)

At: <www.hear.org/pier/index.html>

This website focuses on potentially invasive plant species that threaten Pacific island ecosystems. In addition, there is resource material, such as images and distributions of agriculturally important weeds.

PestNet

At: <www.pestnet.org>

PestNet provides an email network similar to that of the ISSG but is more targeted at agricultural pests. Its purpose is to help plant-protection workers in Southeast Asia and the Pacific. The topics discussed commonly relate to pest identification, requests for specimens and methods of controlling pests.

PestNet has a website that provides information on how to join the email listserver. Follow the instructions on the website for 'Join PestNet' at <www.pestnet.org>. The site also has a photo gallery of numerous pests.

Secretariat of the Pacific Community (SPC), Plant Protection Service (PPS)

At: <www.spc.int/pps>

This group coordinates issues of plant protection across Pacific countries and territories. The PPS focuses on preventative quarantine barriers, preparedness for incursions and management of pests. The site has specific reports on forest pests, their surveillance and management, and a Pacific pest list database.

Traditional Pacific Island Crops

At: <libweb.hawaii.edu/libdept/scitech/agnic>

This website is produced by the USDA's Agriculture Network Information Center (AgNIC) <http://laurel.nal.usda.gov:8080/agnic>. The site contains information on cultivation, pests and marketing issues of numerous Pacific crops such as kava and betel nut. Links to related sites at the University of Hawaii are included.

Enviroweeds

The Enviroweeds list server is moderated by the Cooperative Research Centre for Weed Management in Australia. It is used to distribute and discuss information on the management of environmental weeds in natural ecosystems. To subscribe to Enviroweeds, send an email message to <majordomo@adelaide.edu.au> and in the body of the message type <subscribe enviroweeds>. Do not type anything in the subject line.

2.4.8. Pest information sheets

Pest information sheets provide identifying details of target pests that the survey team can refer to in the field. You might call these sheets a 'field guide'. You will have collected all this information so far in completing this step and so can make your own pest information sheets. These sheets should be simple and easy to read.

A pest information sheet would include:

- the pest's common and scientific names
- host range
- symptoms and morphology
- colour photographs or diagrams of the pest showing the typical morphology at characteristic stages and on multiple hosts (as appropriate)
- preferred habitats—this might include 'unnatural' settings such as plant pots, dunnage (wood packing), market stalls, silos and ship containers
- if appropriate, details of pests that the target pest could be confused with.

Weed sheets would include pictures of the juvenile and mature plants and diagnostic parts such as flowers, leaves and buds in detail.

Step 3

- Record the names of the pest.
- Record the importance of the pest.
- Record the diagnostic characteristics of the pest, including the life cycle.
- Create any pest information sheets you will use in the field.

2.5. Step 4 Identify target host(s)

If host plants are not involved—for example, in the surveying of weeds or pheromone trapping of insects—skip ahead to Step 5.

2.5.1. Host names

List the common and scientific names of targeted host plants.

For forests, list the dominant tree species and common names.

2.5.2. Value of host or commodity

Describe the importance of the hosts; for example, their nutritional value to small communities, and their national or regional economic importance.

2.5.3. Growth habits and life cycle of host plants

Describe the growth habits of each host and any aspects of their life cycle that are relevant to the diagnosis of the pests to be investigated.

List how the host plants of interest are grown; for example, in fields, as a plantation crop, in home gardens, as amenity trees in public spaces.

How tall and bushy does the vegetation grow? How much of the plant could you see and access? Could you collect a specimen from the crown, the middle near the main stem, the tips of the growth, or at the base of the plant?

For weeds, what is the vegetation type in the area to be surveyed?

2.5.4. Accessibility of the host plants

If you are designing a specific survey, consider the vegetation and the areas in which the pest will be surveyed. Information about the accessibility of hosts would be important for a person using your report as part of general surveillance, as it may explain to them why only certain places were surveyed.

Box 3. ISPM quarantine pest categories

regulated pest A quarantine pest or a regulated non-quarantine pest

quarantine pest A pest of potential economic importance to the area endangered and not yet present there, or present but not widely distributed and being officially controlled

non-quarantine pest A pest that is not a quarantine pest for an area

regulated non-quarantine pest (RNQP) A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party (ISPM 5)

RNQPs are present and often widespread in the importing country (ISPM 16).

Comparison of quarantine pests and RNQPs (ISPM 16)

Defining criteria	Quarantine pest	RNQP
Pest status	Absent or of limited distribution	Present and may be widely distributed
Pathway	Phytosanitary measures for any pathway	Phytosanitary measures only on plants for planting
Economic impact	Impact is predicted	Impact is known
Official control	Under official control if present with the aim of eradication or containment	Under official control with respect to the specified plants for planting with the aim of suppression

The remaining organisms would be unregulated (or 'non-regulated'), whether or not they are a 'pest' in some other place or places.

How are the host plants ordered? If they are evenly in rows, could you walk between the rows? Could you see the entire plants in a row if you walked down it (consider potatoes compared to oil palm trees)?

If the vegetation is random, like native forests or market gardens, or even continuously planted, such as broadacre grain, where can you walk or drive? How much damage caused by walking through the crop would be accepted by the property managers? How far do you expect that someone could see into the crop or forest? What is the terrain like? Are there remote parts? Are there any dams, rivers or fences that may affect how you can access the site?

2.5.5. Regional distribution of the host

Describe the distribution of the host in the country/region of interest. List all of the locations by name. For commodity sampling, describe the environment where the commodity will be held during the survey. For example, packing sheds or local markets.

Step 4

- Record the names of the host plants.
- Record the importance of the host plants.
- Record the growth habits of the host plants.
- Record the likely accessibility if considering a specific survey.
- Record the regional distribution of the host plants.

2.6. Step 5. Alternative hosts

The timing of life cycles of other pests and hosts can interact with the pest of interest. Alternative sources of the pest might include other host plants nearby, or in nursery stock or in a seed bank in the case of weeds. These hosts would include *alternate* hosts for fungal pathogens that have an obligatory asexual or sexual life stage on alternate hosts.

Identifying the entire host range is particularly important for early detection surveys of exotic pests as well as delimiting surveys investigating the extent of a pest incursion.

This type of information can again be found from talking with locals, and from publications, databases and resources on the Internet.

Step 5

- Record alternative pest reservoirs.

2.7. Step 6. Review of earlier survey plans

Find out if your colleagues or others in your organisation have designed any surveillance plans. Contact your NPPO and ask the people there if they have any existing plans or can put you in contact with others in your country who have designed surveillance plans. If the plan is connected with trade, the NPPO will need to become involved as part of the process. You could also use the email address lists discussed in Box 2 to seek plans for similar pests or hosts under similar conditions.

These reports may provide you with useful information as you continue to design your plan.

Step 6

- Collect any accessible survey or surveillance plans or reports.

2.8. Steps 7 to 10. Site selection

There are usually six levels involved in site selection (Figure 2).

1. The first is selecting the 'area'. This is *an officially defined country, part of a country or all or parts of several countries* (ISPM 5) that encompasses where you would look for pests.
2. The second is selecting the 'district(s)' involved—these might be growing districts, or regions of the area that appear to fall into rough groups on a map.
3. The third is selecting the 'places' in the districts that could be surveyed; farms, forests, communities, villages, ports or markets, for example.
4. The fourth is selecting the 'field sites' within each place. These could be fields, plantation lots, market stalls (selling the target commodity) or agroforestry gardens.
5. The fifth level is selecting the 'sampling sites' within each field site. This could mean the quadrats, individual plants, trees or produce, transects, trees to which pheromone traps could be attached, or crop rows.
6. The sixth is selecting the 'sampling point', which is relevant when you need to choose specimens within a sampling site. For example, you may have selected 20 papaya trees per orchard as your sampling sites and intend to collect three fruit per tree, or examine the third apical stem from the top. In some instances, such as pheromone baiting or sampling commodities at markets, the sample point would be the same as the sampling site.

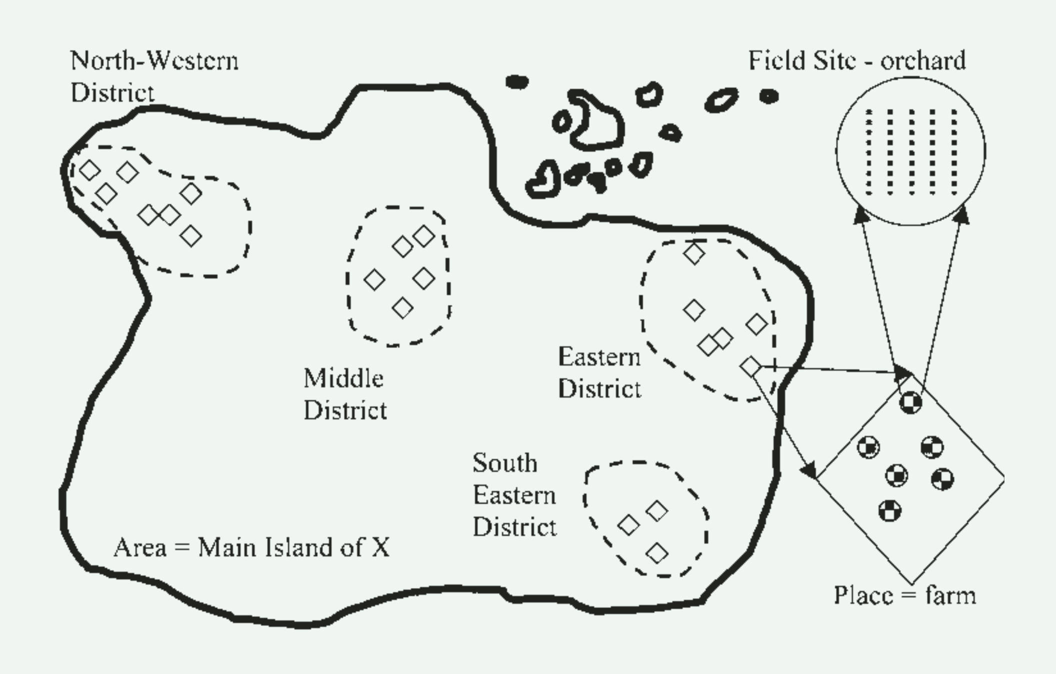


Figure 2. Diagrammatic map illustrating the concepts of area, district, place and field site

2.9. Step 7. Identifying the survey area

The area should be easy to determine. The area is either the entire country or a clearly defined part of the country around which effective quarantine measures can be established.

Step 7

- Record the area for your survey, which will be the same as that recorded at Step 5. Provide brief details on the climate, topography and geographic coordinates.

2.10. Step 8. Identifying the survey districts

If the districts in the area are not known, you will need to research where they are. This may involve speaking to people in the known districts; rangers or government and private organisations that represent particular growers, for example. It may require drawing the places on a map of the area to see trends. Districts may already be known, because they are climatically isolated, for example. There would normally be only one or a few districts and so they should be easy to identify.

Depending on your purpose for surveying, it will be clear to you which districts you need to survey.

Step 8

- Record the district(s) for your survey, clearly identifying each and providing general coordinates.

2.11. Step 9. Identifying the possible survey places, field sites and sampling sites

At this stage, work out what the characteristics of the places, field sites, sampling sites and sample points would be, i.e. what sort of locations they are. Refer to Section 2.7 for examples.

Some surveys will not have sampling sites or sample points, and some may not even require field sites. For example, a person viewing a forest for obvious symptoms from a cliff top could be surveying an entire place.

Step 9

- Record the characteristics of places, field sites and sampling sites.

2.12. Step 10. Methods for choosing sites

Every plan has to include surveying at the place level. This is the minimum level at which a survey can be performed. Some survey types are performed only at this level of site selection. These are surveying from a vantage point (see Section 2.12.3.12) and remote sensing (see Section 2.12.3.13).

Surveys that collect data only at place level are those that look at a large area from a high vantage point allowing the place to be scanned in its entirety. In order to do this, the symptoms or pests need to be obvious at a great distance. As the level of detail is low, surveying from such heights would be inappropriate for most survey purposes, especially those that need to satisfy the detailed requirements of trade partners.

Depending on the reason for your intended survey, you will either already know exactly which sites to survey or you will need to select the sites.

It is worth noting at this stage that there may not be a single best method for site selection. It also may not be possible to use the 'best' method, due to logistical or financial constraints. The main point is to transparently document your choices and reasons for the choices made. These can then be considered and discussed by other parties involved who may well agree with the basis of your choice, given the circumstances.

2.12.1. When you know which sites and how many to survey

Some surveys have to be targeted to particular places, field sites or sampling sites. A delimiting survey is one that involves looking at a pest infestation (so the place and field site are determined by circumstance) and working out how far the pest has spread and how it might have arrived. Delimiting surveys are covered in Chapter 5, but you should continue to work through the steps in this chapter.

In high-risk site surveillance, the places and field sites are determined largely by town planning—those sites where an exotic pest is likely to first appear and surrounding areas, such as sea- and airports. See also Section 2.12.3.1, Targeted site surveillance.

Blitz surveys (see Section 2.12.3.2) are different from all other surveys. They involve choosing a targeted field site (so the place, district and area are already known) and then performing an intensive and fast, 'full sample' at the sampling site level. See also Section 2.12.3.3, Full sampling.

2.12.2. When you need to choose which sites to survey

So how do you choose which sites to survey? Your approach will depend on any constraints imposed on the survey, the likely dispersal of the pest and the type of sampling plan that would best suit.

2.12.2.1. Logistical and physical constraints

The best scenario is being able to look at all places, field sites and sampling sites that are in the districts of concern. In many surveys, this is not possible, because of the costs involved. If you are unable to perform this 'full sampling' (see Section 2.12.3.3), identify your constraints and attempt to quantify these limitations. The point of this is that you may need to work backward and identify how many sample points and sampling sites you can feasibly survey within the limitations you may have on staff numbers, time, money, availability of expertise, weather or other factors. This may involve costing a hypothetical survey (in money and time) and thinking through how the survey would work in practice. This information may then provide clues as to how many locations, places and districts you could survey.

2.12.2.2. Pattern of spread of the pest

If you assume that the pest is present in the area of interest, how would the pest spread or be dispersed? Understanding how the pest spreads across a crop or other sites will affect how specific surveys are planned. This is also relevant to general surveillance when interpreting reports of specific surveys that may be used as a source of information.

Pests such as flying locusts will spread randomly throughout a crop, while others, such as nematodes and some weeds, tend to clump in small areas of the field. Pests may also prefer particular aspects of an area, such as along a watercourse or fence line.

If the pest is expected to spread randomly, or that clumps of pests will be randomly distributed, then sampling anywhere in the field should give you an equal chance of detecting the pest. This is important when you cannot observe all of the sites.

If the pest tends to prefer a particular area of a crop, then this area may need to be specifically targeted in the sampling plan (see Section 2.12.3.1, Targeted site surveillance).

2.12.2.2.1. What if the distribution is unknown?

If the pest is present, a preliminary inspection during a pilot study (Step 18) can be performed. The landowners and farmers may also have knowledge of any patterns of concentration of pests.

2.12.2.3. Surveying all sites

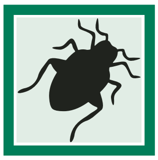
If you choose to do all the sites at any one level, this is called full sampling of that level. Full sampling provides the most detailed data of all the survey types. More information on full sampling can be found at Section 2.12.3.3.

Another source of robust data can sometimes be people who work at the field sites. If there are people available who are very familiar with the site and the targeted pests, they may be able to narrow the search. See also Section 2.12.3.4, Crop/forest worker observations.

2.12.2.4. Surveying some sites

If you cannot attend all sites at each level, you will need to select which ones to attend. To do this you can use one or a combination of four tools.

1. The first is random sampling. This involves assigning all sites (of the same level) a number or symbol and then by using a random number generation method, the sites are selected and recorded. See Section 2.12.3.5, Random sampling surveys.
2. The second is systematic sampling. This involves selecting criteria to divide the sites into some form of regular intervals and then selecting on that basis (see Section 2.12.3.7, Systematic sampling surveys). For example, surveying every second site when listed by name in alphabetical order, setting up a grid of traps or parallel transects of a site.
3. The third is stratification, which can be used in combination with random sampling. This involves dividing the sites into logical categories and then systematically or randomly choosing sites from within the categories.
4. The fourth is targeted site selection. The sites are chosen based on where the pest is most likely to be, thereby deliberately biasing the selection process in favour of finding the pest. See Section 2.12.3.1, Targeted site surveillance.



> Surveys should normally be designed to favour detection of specific pests concerned. However, the survey plan should also include some random sampling to detect unexpected events. It should be noted that if a quantitative indication of the prevalence of a pest in an area is required, the results from targeted surveys will be biased and may not provide an accurate assessment.
>
> **ISPM 6**

For more information on bias, read Box 4.

There are some other methods that people use to select sites but the methods introduce selection biases and do not have an element of genuine randomness.

The first is haphazard sampling (see Section 2.12.3.9), in which a person tries to select (for example) places randomly without using independent random number generation methods.

The second is convenience sampling (see Section 2.12.3.10). This involves selecting sites according to ease of access, such as those closest to a road. This method is often used in forestry when large distances may need to be covered, and is termed a 'drive through' or 'walk through' survey (see Section 2.12.3.11). It can be used in conjunction with additional detailed surveys in selected sites.

Other survey designs that do not involve randomness, but are nonetheless valuable tools for assessing large areas of crops or forests, are viewing from a high vantage point (see Section 2.12.3.12) and remote sensing (see Section 2.12.3.13).

Step 10

- Record method for choosing places to survey.
- Record method for choosing field sites to survey.
- Record method for choosing sampling sites to survey.
- Tabulate all possible places, field sites and sampling sites being considered, providing these with individual identifiers.

As mentioned above, you may at this stage already know how many sites at each level to survey. If this is the case, go to Step 12 Timing of the survey.

If you have chosen a method, such as random sampling to select sites, you will now need to work out how many of these to survey. Go to Step 11 Calculating sample size.

2.12.3. How survey types affect site selection

2.12.3.1. Targeted site surveillance

Targeting particular sites is designed to maximise the chance of finding the pest.

Surveillance for early detection of exotic pests usually involves targeting sites that are the first point where exotic pests could arrive or infest. Goods and people that may carry pests enter a country by crossing borders or arriving at sea- or airports. Some pests can travel on the wind or down waterways that could cross between countries or islands. Depending on the possible routes of arrival, these sites are targeted for surveillance. The intensity of survey sites is highest around the first points of entry and then is reduced in frequency with distance.

Targeting can also be in the field or forest where surveillance is focused on host plants or sites where the pest is most likely to be present (and thereby deliberately introducing bias). This might include surveying fruit that ripen or drop early or are rejected in the packing shed; or areas in the field adjacent to a creek.

Field workers, property managers and others working at the places of interest, may be able to provide local knowledge of where any pests present may have been observed. This could identify particular niches where the pests could be found.

Advantages

- Useful for early detection of exotic pests.

Disadvantages

- Of limited value in providing information about the prevalence of the pest.

Box 4. More information on biasing the results

When samples or observations have been collected, recorded or interpreted in a way that consistently affects the data, either by overestimating or underestimating the actual number of pests, this effect is called bias and causes error in the results. This can easily happen in a number of different ways and, in some survey designs, aspects of site selection are deliberately biased. Deliberate biases may be introduced when the survey designer is trying to select the sites where the pests are most likely to be, rather than work out what the prevalence is over a large area.

In situations such as determining pest prevalence and investigating whether or not an area is truly free of a pest, it is important—in order to collect accurate information—to prevent as many causes of bias as possible.

Selection bias

It is easy to select a plant or site based on the characteristics of the sites that are being surveyed. Some symptoms or weeds are easily visible from a distance and naturally draw the eye toward these areas. Consciously or unconsciously, a person may head towards or away from pests. A person may want to avoid difficult or tedious locations, or tire of repetitive searching.

Counting bias

This bias results when counts, say pest per square metre, are consistently less or greater than the true number because the person involved consciously or unconsciously prefers a low or high score. This can be worsened if more than one person is involved in the counting and each person has a different counting bias. The capacity to identify a pest or its symptoms may vary between people inspecting sites.

Recall bias

Bias can be introduced when a person records data on a pest based on memory of earlier observations. Errors can result from not remembering accurately where, when or which pests were present or absent. These biases can be reduced by the collection of a specimen when possible and recording details at the time of observing the pest or symptom. If this is not possible, the recalled observations will need to be confirmed or treated with a degree of caution.

Sampling error

Sampling error could arise, for example, from any of the following circumstances: when insects on a plant are disturbed and then cannot be counted; where weather influences the count, e.g. leaves hang differently when wet; because of differences in pheromone plume carriage; or failures in collecting equipment. Errors from assessment arise when there are faults in measurement, such as incorrectly calibrated instruments, setting the traps to capture insects at times that do not show peak numbers or placing them too close together or too far apart, variation between different people's counting methods and diagnostic capacities, using the wrong pheromones, or inability to use equipment correctly or to handle samples appropriately.

2.12.3.2. Blitz surveys

The purpose of blitz surveys is to detect all pests present, even those in low numbers, and to identify less visible symptoms and newly emerging pests. These surveys involve the intensive inspection of all plants in a given field site or at a set time, generating pest lists for a host or range of hosts. The survey may be restricted to a list of pests that have particular relevance or risk. Blitz surveys are generally used only in high-risk areas such as ports. The surveys require a range of specialist botanists, entomologists and pathologists to be involved in identifying the weeds, insects or pathogens of interest. The effectiveness of blitz surveys to identify new pests depends on the vegetational structure—for example, surveillance of large trees is difficult, particularly for pests or symptoms that affect the tree crown—and on the resources and expertise of the specialists to diagnose the pests.

Advantages

- Provides high confidence about the pest status in a small area.
- Can be used to determine the pest prevalence in the area.

Disadvantages

- Information is restricted to a small area.
- Can be expensive or difficult to coordinate, particularly organising the involvement of numerous experts.

2.12.3.3. Full sampling

Full sampling involves examining all the sites at a particular level. This could be full sampling of all places right through to surveying all sampling sites at a field site. This term overlaps with blitz surveys which entail full sampling at the field site level.

Advantages

- Sampling all units means that there is no selection bias in the sampling plan and provides a high confidence in the data.
- Can be used to estimate prevalence and as part of early detection of pests or in monitoring surveys.
- If there is a low predicted prevalence of the pest, this type of survey will detect any pests present.

Disadvantages

- Full sampling has limited application, as often it is not possible to survey all host plants, sites or regions because of financial and logistical constraints.
- Full sampling may not be the best use of resources if, for example, there are many fields that could be surveyed, and only a few are surveyed in full. Resources would instead be better spent surveying fewer host plants per field and visiting more fields, as there may be wide variations in field-to-field prevalence of the pest.

2.12.3.4. Crop/forest worker observations

In this case, people who manage crops or forests report to a central person, say the property manager, pests that they have seen during their work. The workers must recall where, when and what they observed. Alternatively, landowners show surveyors where they have observed pests or diseased plants. Given an understanding of the closeness of the relationship between the observer and the plants and area involved, the information may save a great deal of surveying for early detection of pests. In these situations, it is very important that field workers be well informed of what the surveyors want to know.

Advantages

- Economical because the surveying is performed during other activities.
- The quality of data may be equivalent to a full survey if the workers are very familiar with the sites and pests, and especially if they have knowledge of the sites over time.
- Can be valuable in the detection of new pests.

Disadvantages

- Cannot provide a quantitative measure of prevalence unless the prevalence is low and obvious.
- The timely detection of pests relies on the frequency of the activity that brings workers to a site. This may be too infrequent; for example, in forests with difficult terrain.

2.12.3.5. Random sampling surveys

Usually, all sites and host plants cannot be examined and so a subset number of sites or host plants need to be chosen for surveillance. To avoid selection biases, all hosts and sites need to be equally likely to be surveyed. In random sampling surveying, the sites and plants are chosen by an impartial method that reduces the influence of human biases in the site selections. These impartial methods—methods to introduce randomness into a survey plan—are detailed in Box 5, page 42.

Systematic sampling (see Section 2.12.3.7) can also be viewed as having a random element if the intervals of the sampling are independent of the expected pest distribution. For example, regularly spaced sites should not coincide consistently with the presence or absence of the pest.

Advantages

- As the selection of sites is independent of the pattern of pest spread, a random element may detect pests where other survey designs might not. Because of this, the ISPM recommends that all survey plans *should also include some random sampling to detect unexpected events* (ISPM 6).
- Can be simple to introduce randomness into a plan.
- Can be used to determine pest prevalence as part of detection or monitoring surveys.

Disadvantages

- May lead to impractical site choices or order of sites to be visited and may need to be combined with other methods, such as stratification of higher levels than those randomised.
- Randomisation of sites may miss clustered pests, and may be frustrating if the pests are visually obvious and the survey design is committed to randomly selected sites that all miss the pests. (In this instance, you would reconsider the design choice.)
- There are some aspects of sampling that cannot be randomised. For example, trees in an orchard can be randomised as they are fixed in number and location. The selection of fruits on each tree cannot be randomised (before going to the field) as each tree will vary in the number and exact location of branches, leaves or fruit (etc.) on the tree. However, even in this case, a dice could be thrown where the numbers specify branch number from top or bottom or a hypothetical slice/portion of a plant. With a little imagination, randomness could be added to most elements of the sampling site selection process if needed.

2.12.3.6. Stratified random sampling

In stratified random sampling, the host plants or sites are systematically divided into groups and sites or host plants are randomly chosen within each group.

Example: 20 villages (level: place) are to be surveyed for banana diseases. Each village has 15 farms (level: field sites), a total of 300 farms. If 100 farms are to be surveyed, we could randomly choose the 100 from all 300. By chance, this may result in some villages having all their farms surveyed and others having none. If it is important that all villages be surveyed, the selection of the 100 sites can be stratified by village such that, for example, five farms per village are chosen randomly.

Advantages

- Provides a tool that allows an often practical element to be mixed with random sampling.
- Can be used to determine pest prevalence and as part of detection or monitoring surveys.

Disadvantages

- If the distribution of the field sites (e.g. number of farms per village) varies widely, sampling an even number from each farm may not show the true prevalence, as there would be an uneven distribution of the possible host sites. In this case, the selection of sites may need to be 'weighted' toward those places (villages) with more field sites (farms).

2.12.3.7. Systematic sampling surveys

Systematic surveys involve mapping out a site and surveying at regular intervals of distance, area or host plant. For example, examining the plants of every tenth row; every third farm; every eighth square metre; setting insect lures in a grid pattern; two apples from every tree; or performing parallel sweeps of a site.

Advantages

- It is simple and efficient.
- The sample number is proportional to the population size.
- It may not be necessary to count the entire population (i.e. to know exactly how many rows there are in all crops to survey) before developing and performing the survey plan.
- Survey staff have clear sampling instructions to follow.
- For pests with a clustered distribution, a systematic survey can provide a better chance of detecting the pest than can a random sample. This is because a random sample may completely miss even a large cluster that a systematic survey with close intervals would detect.
- Has a random element if the intervals are independent of the pest distribution.
- Can be used to estimate prevalence in monitoring surveys.

Disadvantages

- Difficult to use if the hosts are not growing in an ordered pattern or all areas are not equally accessible.
- Need to ensure that if the survey is subsequently repeated in the same locations, the same plants or square metres are not surveyed repeatedly. This could be achieved by moving the starting point (e.g. by one row) each time the survey is performed.

Box 5. Adding random elements into the survey plan

The 'W' sampling strategy and diagonals

Walking and examining hosts or square metres of soil in a very large zigzag pattern across the field or forest can add a random component to the sample sites chosen. Crossing the field diagonally, or in a large, inverted W pattern serves the same purpose. One problem with this approach is that if the field is to be surveyed more than once, then not all plants in the field have an equal chance of being examined and the same plants could be looked at repeatedly. Rotating or offsetting the starting point or direction to the field of the W, diagonal or zigzag could overcome this problem, providing there is little overlap.

Random number generation

Randomising the order in which sites are visited can be achieved by assigning each site a sequential number and carefully listing the sites and their numbers. It may be important to record the order in which the numbers are chosen because for some surveys, such as species-accumulation curves, this will determine the order of the sampling sites you will survey.

Generating a list of random numbers

Using dice, thrown objects, card decks and numbered pebbles

If there are only a few sites, randomly choosing the sites or order for sites to be surveyed can be achieved by rolling dice and recording which numbers come up and ignoring repeated numbers.

Alternatively, cards labelled with site numbers or names can be well shuffled and read off in the order they appear. Clearly, the cards must be very well shuffled as cards can clump and shuffle in groups, and the same sites could be selected more often than others. These methods are useful unless the number of sites is more than a literal handful of cards.

Another method is to stand at different positions in the field and throw a stick (or something that is visible and will not damage the crop). This method will be influenced by the individual's throwing strength, and if the object can be found. Throwing the object backwards may reduce the chance of throwing in a deliberate direction.

Pebbles numbered with a marking pen according to numbered sampling sites can be mixed and selected at random. Other items to hand, of reasonably uniform size, that could be numbered and mixed up could be equally useful.

Using Microsoft Excel on a standard computer

Work out the range of your site numbers. You may have 92 sites numbered 1 to 92.

Method one

Select a cell in a worksheet type in the function RANDBETWEEN. This function will generate numbers between a range that you choose. In this case, between 1 and 92. The equation would be

= RANDBETWEEN(1,92)

On pressing <enter>, a number between that range will appear. Copy and paste this formula into as many cells as you need, recording the numbers as they appear and skipping numbers that have already appeared. Record the number of random site numbers that you need. If this function does not work on your computer, use the Help feature of the program.

Method two

This method overcomes duplication. Using the example above, create a column containing numbers 1 to 92 in sequence. In the adjacent column cells, type =RAND( )[6] against the 92 cells. Select all the cells in both columns and <sort> (on the Data menu) using the column containing the random numbers as the sort column. This will sort the column containing the numbers 1 to 92 randomly, without any duplication. Then you can simply take as many numbers as needed working from the top.

Using the Internet

There are many sites on the Web that have tables of random numbers, or programs that generate random numbers which can be downloaded, but if you have access to the Internet, chances are that you will have access to Microsoft Excel and be able to create your own random number table. As website addresses often change over time, none are listed here. A simple search of the term 'random number table' will be sufficient to find useful sites.

Using random number tables

Tables of randomly generated numbers can be found in print. Essentially these tables are generated using programs such as that described above, randomising numbers between 00001 and 99,999, to get sets of five-digit numbers. A table has been provided on the next page. You can use the numbers across or down the page. If we continue the example above in which the highest site number is 92, which is a two-digit number, we read the numbers in sets of two digits and ignore numbers that are single digit and those that are less than 1 or greater than 92. The numbers 1 to 9 are preceded by a 0, i.e. 01 to 09. For example, the first row of numbers is:

56888 17938 03701 19011 21795 81858 84375 52174 30547 01838

This is read as 56 then 88, ignore number 8 as it is a single digit, then number 17, ignore number 93 as higher than 92, ignore the number 8, then 3 and 70, ignore the 1 and so on until enough sites have been chosen. The next time that you require random numbers, start somewhere else in the table, read down the rows or even read the numbers backwards.

---

6 Do not insert anything between the brackets

If you are choosing from a three-digit number, e.g. you have a total of 480 sites (so numbers between 001 and 480), read the first three digits and ignore the fourth and fifth of each random number, i.e. ignore 568 as greater than 480, ignore the 88, record 179, ignore the 38, record 037, ignore 01, then 190, ignore 11, then 217, ignore 95 and so on.

Latin squares

Another simple method for introducing a random component into the sampling plan works by assigning sites a number or letter. The order in which sites are observed is the same but the starting site at each successive survey changes.

This might be useful to reduce any bias that results from time including season. It is usually used when all the sites can be rotated through regularly.

e.g.	Visit sites in this order:				
time point 1:	A	B	C	D	E
time point 2:	B	C	D	E	A
time point 3:	C	D	E	A	B
time point 4:	D	E	A	B	C
time point 5:	E	A	B	C	D

Random number table

56888	17938	03701	19011	21795	81858	84375	52174	30547	01838
49616	05027	58559	77518	88818	15510	05166	17778	45383	63979
87810	50654	12571	64281	18565	63604	97574	77022	10497	70113
77768	24763	85849	17644	59367	55704	67362	91953	87927	54886
15685	77153	56972	83849	91933	04399	54762	71614	87482	66997
57092	05782	67929	96388	87619	87284	16247	86247	68921	61431
45805	97856	91292	58860	19103	04612	88838	39043	28360	38408
52092	41346	76829	28270	42199	01882	43502	20505	92532	87558
78094	24397	88649	24778	14083	25737	96866	53011	60742	04056
42069	88809	18431	08841	19234	28425	08699	86805	11950	71287
88748	65229	69696	94302	99033	64739	41696	46127	05953	25836
77027	57205	73195	17923	13149	23871	64516	54129	60723	12240
14727	32085	97754	87565	68544	47424	18127	39214	31843	50282
67741	79843	97622	21539	83690	87439	42371	92319	95824	77041
73620	81275	57875	76408	47690	23760	67511	71723	86944	46318
27839	40135	78953	09577	70296	79014	72997	52780	62760	34873
81980	85841	90030	81070	98649	97659	10671	89893	21450	57957
63538	95903	70908	23910	57908	67982	27523	62498	27636	02209
34182	62714	03756	64533	26160	20042	11142	00536	93365	08796
30918	27213	10699	59679	59136	82891	77801	62105	81536	91477
85473	23571	50458	11012	03006	83667	68269	23315	18286	48988
53811	39465	95669	80783	34150	65472	90418	48305	32304	23130
90354	51729	98512	79972	29695	38245	38004	81201	31328	38571
75420	48164	33446	07120	13909	10215	51857	19984	41887	17670
00454	95064	31329	06519	85296	07531	22075	30769	73421	17858
61307	17016	64835	16959	47499	42525	38932	33886	48382	88842

2.12.3.8. Insect-trapping surveys

Insects can be caught by static traps that attract insects by light, colour or pheromones. The insects are then removed from the trap and identified. These traps are useful primarily for identifying whether or not a pest is present in the area.

The siting and density of traps is critical. Siting and density are determined by the trap type and the manufacturer's instructions, and applied according to the survey setting.

Traps are often used to estimate the prevalence of pests in the area. In some cases, the number of insects trapped is directly proportional to the true pest prevalence (e.g. 1 trapped fly could reflect 100 flies in the area).

Advantages

- Once set up, the traps can be left unattended for weeks.
- Very useful in the early detection of attracted pests.
- Placement of traps need not damage the crop or forest.
- Can be used as an indicator of the pest prevalence.
- Traps with selective lures help to target the catch to specific pests.

Disadvantages

- Some traps can fill with rain or have other design problems that may need to be managed.
- Traps can attract pests from outside the targeted area or from plants in nearby crops or native vegetation. This can cause difficulty in interpreting the catch. In this case, the host range for each species may need to be checked to ensure that the pest interacts with the targeted host.
- Counting and identification of pests from traps can be time-consuming and laborious.
- Using the number of pests collected as a quantitative measure of prevalence or density is complicated because of the many variables involved.
- If not set up with the correct density and positioning, pests that are present can be missed.
- The selectivity of lures can be restrictive when surveying to determine the entire range of pests present.

2.12.3.9. Simulating randomness—purposive and haphazard sampling

Purposive sampling involves choosing places, field sites, sampling sites or even sample points that the observer decides are representative of the whole site. This is based on the observer's preconception of what the pest status is and the observer will, consciously or unconsciously, be biased toward fulfilling that preconception.

Haphazard sampling is the term for observers attempting to collect 'random' specimens by mentally selecting sites sporadically. There is, nevertheless, a tendency for people to distribute sites uniformly, or choose sites based on an idea of a 'randomised' pattern. For example, people generally would not consider choosing clustered sites within a large area, and yet such a configuration can result if the sites are chosen randomly. If the sampling points are chosen in the field, rather than pre-selected from a map, the observer's eye tends to be drawn to certain plants or symptoms. The observer is then faced with a quandary: is the sampling truly random if these sampling points are consciously included or consciously excluded? In most situations, it is impossible for a person to truly simulate random sampling.

Advantages

- May be useful in circumstances where true randomisation is not possible.

Disadvantages

- Introduces biases into the data that may compromise the outcome.
- Cannot be used to estimate pest prevalence.
- May be unable to detect new pests in a timely manner.

2.12.3.10. Convenience ('rule of thumb') sampling

Sites are selected that are easy, quick or inexpensive to survey because, for example, they are close to each other, close to a road or access point, topography is easiest, or because a tree has lower branches or more fruit than others.

Advantages

- The method is convenient and rapid.

Disadvantages

- This approach has selection bias.
- Cannot be sure how representative the data are of the whole field site.
- Has no element of randomness.
- Cannot be used to estimate prevalence or to detect changes in pest populations or as a reliable early detection survey.

2.12.3.11. Drive/walk through surveys

In this method, one or two people drive a car, walk or ride a bicycle or motorcycle around or through an accessible part of the crops or forest, looking as far as they can see for any obvious pests or symptoms. They may stop and collect specimens if a pest or symptom can be sampled. The reliability of this type of surveillance is dependent on the skills of the observer, the density and height of the vegetation and of the symptoms or pests, the topography and how representative the visible areas are of the entire crop or site. In optimal conditions where obvious symptoms are being surveyed, driving speeds should not exceed 15 km per hour. In such circumstances, an observer could not be expected to see reliably more than 40 metres away (unless they are along a high vantage point).

Advantages

- Provides the 'quick once over' perspective of obvious symptoms.
- Does not damage the crop or forest being surveyed.
- May be useful in targeted surveillance or early detection of pests when the pests are spread by vehicles and people, and so are likely to be found on road verges.

Disadvantages

- Cannot provide a measure of pest prevalence.
- Cannot provide information on pests or pest symptoms that are difficult to see.
- The surveying perspective is restricted to accessible paths and roads.
- Could be dangerous to the surveyors if the drivers are not watching where they are driving.
- Dependent on the layout and number of roads at the site.

2.12.3.12. Viewing from a high vantage point

This procedure involves viewing the landscape from a high point such as on the top of a hill or the side of a valley. Binoculars can be used to increase effectiveness.

Advantages

- Information on large areas can be collected in a short time.
- Provides a 'quick once over' perspective for obvious symptoms.
- Allows the crowns of trees or other tall host plants to be seen.
- Permits surveillance of terrain that is difficult to cross on foot or by vehicle.

Disadvantages

- The symptoms or pests need to be highly visible, which may mean that the pest is well-established and has already caused significant damage.
- Cannot be used to accurately determine pest prevalence and/or for very early detection of a pest.

2.12.3.13. Remote sensing

Remote sensing is an umbrella term for methods of surveillance that are performed high above the ground, either at the altitude of an aeroplane or of a satellite—sensing the landscape from a remote perspective. Remote sensing works on the basis that the pest or host symptoms of interest are distinct in appearance from adjacent vegetation. The images of the vegetation are captured using sensors, such as specialised cameras or radar, and the image can be processed by computer programs. The programs can produce maps of vegetational types present and perform calculations such as the percentage of an area infested by a pest. Remote sensing has been used to detect and monitor damage caused by insect pests and plant diseases, as well as the presence and spread of invasive plant species.

For further information see:

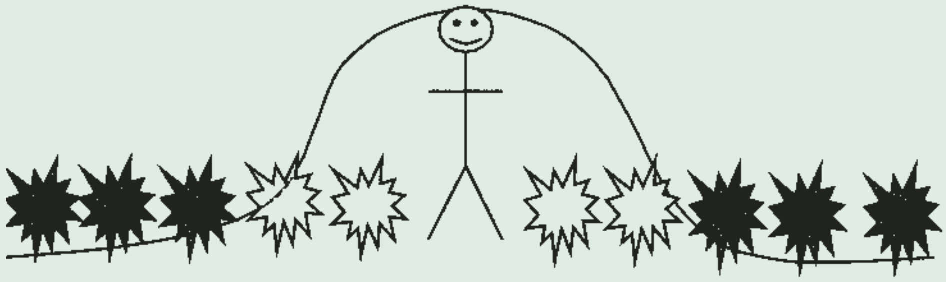
Greenfield, P.H. 2001. Remote sensing for invasive species and plant health monitoring. Detecting and monitoring invasive species. Proceedings of the Plant Health Conference 2000, 24–25 October, Raleigh, North Carolina, USA.

and the following journals:

International Journal of Remote Sensing
Photogrammetric Engineering & Remote Sensing.

Box 6. Validating data collected by viewing from a distance

When walking or driving in a straight line, a person is more likely to see pests or symptoms that are close by. As distance increases, there is less chance of them being able to see the pest or symptoms. Reliability is further influenced by the observer's height, visual acuity and speed of travel, weather conditions and the density of vegetation.



The ability of a survey team to detect pests and symptoms at various distances can be evaluated by placing artificial 'pests' at different distances along a pathway (or road etc.) and at different distances from either side of the pathway. The person placing the 'pests' records the distance of each one along and away from the path. Survey members then walk or drive along the path and record the distance along the path that they observe each of the 'pests'. The artificial pest should be something that simulates the size and appearance of the pest or symptom that will be surveyed, such as artificial frass (insect droppings) made from sawdust and glue that can be pinned to trees. The observations can then be collated for the team as a whole to work out at what distances from the path that the reliability of pest detection falls.

The tests could be repeated a number of times with the artificial pests being placed at different distances until enough data are collected.

Factors that may influence how effective team members are at detecting the pests will be the duration they have been surveying in the field on the day, weather conditions, the number of pests set out, the height of the pests and how obvious the pest or symptom is. Identifying factors that affect how the team perform could then be investigated to increase performance. For example, having breaks every 2 hours.

Advantages

- Information on vast areas can be collected in a short time.
- Can provide a crude estimate of prevalence.

Disadvantages

- Limited application as it can be costly (such as accessing satellites).
- Provides very general data.
- Will work only for pests or symptoms that are easily distinguishable from healthy adjacent vegetation.

2.13. Step 11. Calculating sample size

The purpose of a survey is the primary factor in determining the way sample size is calculated. The two approaches considered here are for detection surveys or monitoring surveys.

This section assists with calculating sample size for settings where you need to calculate the proportion of sampling sites or sampling units infested with pests; for example, either the pest is present on a fruit or tree or is absent. It does not deal with estimating a sample size to ensure an accurate measure of the *concentration* (or population density) of a pest, i.e. the number of pests per fruit or tree.

To calculate the sample size for your survey, there are parameters that need to be understood at least in concept. This step provides some basic calculations that you can perform, but statistics very quickly become complicated and this is when you may need to involve a mathematician or statistician who understands your statistical requirements. Once you have an understanding of the basic parameters explained here, you will be better prepared to provide the information that the statistician will need and have a better understanding of the outputs that they provide you with.

For more detailed information, you will need to read publications such as:

Binns, M.R., Nyrop, J.P. and van der Werf, W. 2000. Sampling and monitoring in crop protection. The theoretical basis for developing practical decision guides. CAB International, Oxon, UK and New York, USA.

This publication is written for people well-versed in mathematical statistics.

2.13.1. Statistical parameters for sample size calculation

The main parameters (expressed in percentages except for sample size which is in whole numbers) are as follows:

2.13.1.1. Actual prevalence

This is the true proportion of infested units in a population (infested by one or more pests).

2.13.1.2. Design prevalence

This is usually based on a pre-survey estimate of the likely actual prevalence of the pest in the field, and used to determine the sample size.

Clearly, for area freedom, the design prevalence and actual prevalence of a pest are expected to be near-zero. For surveys that monitor a pest that is known to be present, the design prevalence can range from near-zero to 100%.

If the design prevalence greatly overestimates the actual prevalence, the sample size calculated will be too small to detect the actual prevalence. If the design prevalence underestimates the actual prevalence, then the sample size will be larger than needed to detect the actual presence, leading to over-sampling. While over-sampling wastes resources, it is safer to over-sample than under-sample.

So how do you estimate the design prevalence? Even if it is near-zero, this parameter needs to be quantified. There are a number of ways to do so; see Box 7, Predicting prevalence. If you are unable to predict a meaningful prevalence, you need to choose a prevalence level that is acceptable to all parties.

2.13.1.3. Estimated prevalence

This is the prevalence determined during a survey, and is intended to estimate the actual prevalence.

The estimated prevalence found during the survey may not reflect the actual prevalence because of factors such as use of methods that have a poorer accuracy or sensitivity than was known or accommodated in the survey plan, or choosing a survey design that does not provide a true sample of the pest.

2.13.1.4. Confidence

Statistical confidence is the probability that the actual prevalence will be within range of the design prevalence.

If you surveyed and did not find the target pest, you cannot be 100% certain that it is not there without sampling every host plant or sampling site. Instead, you have to accept a degree of uncertainty about the plants or areas that have not been examined or tested. The relationship between confidence and sample size is simple—the more sites you survey, the more certain you can be about the accuracy of the estimated prevalence.

As a general rule, a detection threshold of at least 95% confidence is considered acceptable. Confidence up to 99.9% can be necessary in some instances. In some circumstances, choosing the confidence level will not be up to you. Trading partners may require a particular level of confidence that the pest would be detected in a survey, independent of any logistical or financial constraints.

Confidence is usually expressed as an interval of values for the prevalence, forming a range of values within which the actual prevalence is likely to occur with the chosen level of confidence. For example, a prevalence of 46.5% with a 95% confidence might be expressed as: 46.5% (95% CI: 44.2–48.8%).

The range of values is usually of equal ‘width’ (distance) from the prevalence and is termed the *confidence interval width*.

2.13.1.5. Accuracy of methods (sensitivity)

This deals with how well the survey will detect a pest when it is present.

Diagnostic methods used to classify the sample as positive or negative, particularly those involving chemical reactions, often have an estimate of how accurately the method detects positive results. For example, method accuracy can be altered if you are looking for pests on a row of trees but are inspecting only along a straight line. It is expected that the observer cannot see all of the trees if the foliage is dense or the symptoms or pests are not obvious (see Figure 3). An amount such as 80% can be specified as the accuracy of this method. Some methods can reasonably be expected to be 100% accurate. Method accuracy has a direct effect upon the ability to detect the presence of a pest and must be considered when estimating sample size.

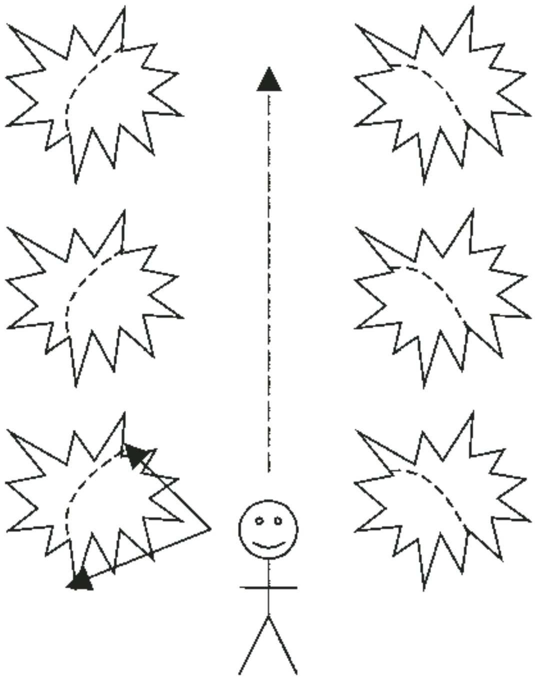


Figure 3. Straight line inspection of trees

2.13.1.6. Sample size

Sample size is the number of sites that you need to survey in order to detect a specified proportion of pest infestation with a specific level of confidence, at the design prevalence.

2.13.2. Formulas for detection surveys

These formulas are used when the survey is designed to detect a pest, and where the actual prevalence is likely to be rare.

A simple relationship exists between sample size, confidence level and detection threshold, where confidence is expressed as a percentage and detection threshold on a scale between 0 and 1.

Formula:

$$\text{Confidence level} = 1 - (1 - \text{design prevalence})^{\text{sample size}}$$

and therefore

$$\text{sample size} = \frac{\log (1 - \text{confidence level})}{\log (1 - \text{design prevalence})}$$

Table with calculations performed:

Table 1. Sample size without method accuracy adjustment

Confidence	1 – confidence	Design prevalence	1 – design prevalence	Sample size
0.95	0.05	0.01	0.99	298
0.95	0.05	0.02	0.98	148
0.99	0.01	0.01	0.99	458
0.99	0.01	0.02	0.98	228
0.95	0.05	0.001	0.999	2,994
0.95	0.05	0.002	0.998	1,496
0.99	0.01	0.001	0.999	4,603
0.99	0.01	0.002	0.998	2,300

If the method accuracy is less than 0.95, the sample size will need to be adjusted. Use the following formula.

$$\text{Adjusted sample size} = \frac{(\text{sample size above})}{\text{method accuracy}}$$

Box 7. Predicting prevalence

When the design prevalence is anticipated to be near-zero (detection survey setting)

To predict the pest prevalence, you need to estimate a 'pest prevalence start date' from when the pest could possibly have last entered the survey area. This date could be from when quarantine measures were put in place to prevent the pest entering the area. Alternatively, it could be when a pest was last officially eradicated from the area. At this time, it is assumed that a tiny number of the pests remained, so at the start date the prevalence is very low.

Next estimate the rate at which a small population of the pest would multiply and spread over time in the area as a percentage of the hosts/sampling sites affected. This is based on the pest's rate of multiplication, spread and survival. All predictions need to have some supporting documentary evidence. You then make a prediction of what the prevalence would be at the time you intend to survey.

When the predicted prevalence is thought to be greater than near-zero (monitoring survey setting)

Generally, if you know that the pest is present at the field sites you intend to survey, there will be data or anecdotes available about the prevalence of the pest at some time point. You may need to take into account how the time of survey relates to the pest's and host's life cycles, and any other conditions that may affect the prevalence, such as weather conditions. This activity falls under the category of *predicting by extrapolation* as detailed below.

Tools to help predict prevalence

Predicting by extrapolation

This is based on the observed rate of infestation of the same pest elsewhere, or in the same location before its previous eradication, allowing for different environmental conditions, through use of reports in journals, field observations and trials.

Predicting by comparison

This is based on the prevalence of a pest with similar population dynamics.

Predicting by modelling

This uses knowledge of the rates of infestation and spread under the conditions present since the 'pest prevalence start date'. It may involve complex computer modelling if there are no other useful or comparable sources of the likely pest prevalence.

Table 2. Sample size with method accuracy adjustment

Confidence	Design prevalence	Method accuracy	Adjusted sample size
0.95	0.01	0.80	373
0.95	0.02	0.80	185
0.99	0.01	0.80	573
0.99	0.02	0.80	285
0.95	0.001	0.80	3,743
0.95	0.002	0.80	1,870
0.99	0.001	0.80	5,754
0.99	0.002	0.80	2,875

2.13.3. Formula for monitoring surveys

Example scenarios:

1. Estimating the proportion of trees in an orchard or forest stand that is infested with a pest.
2. Estimating the proportion of fruit with a pest present.
3. Estimating the number of orchards infested with a pest.

The formula below is used when you choose to have 95% confidence and the expected prevalence is greater than 2%. This uses a variable called 'Z'. 'Z' is derived from the normal distribution and equals 1.96 for 95% confidence, used in the formula below. Note that for 99% confidence, 'Z' is 2.58 and for 90% it is 1.65. Confidence interval width and prevalence are expressed as a decimal between 0 and 1 for the formula:

sample size = (Z/confidence interval width)^2 × design prevalence × (1 – design prevalence)

For example, when the confidence interval width is within 5% and a design pest prevalence of 20% is selected:

sample size required = $((1.96/0.05)^2 \times 0.2\ (1 - 0.2)) = 246$

Table 3. Example sample size calculations performed with a 95% confidence level

Confidence interval width[7]	Design prevalence					
	2% or 98%[8]	5% or 95%	10% or 90%	20% or 80%	30% or 70%	50%
± 1%	753	1,825	3,457	6,147	8,067	9,604
± 2%	188	456	864	1,537	2,016	2,401
± 5%	30	73	138	246	323	384
± 7.5%	13	32	61	109	143	170
± 10%	8	18	35	61	81	96
± 15%	3	8	15	27	35	42
± 20%	2	5	9	15	20	24

2.13.4. Determining sample size for multiple levels of site selection

Calculating sample size quickly becomes complicated when you need to determine many sites at more than one level. For example, you may have thousands of field sites to choose from as well as too many sampling sites at each field to be able to survey all of them. You may even have too many places to survey. These situations call for a hierarchical analysis of the number of sites to visit at each level. Such an analysis sequentially takes into account the number of sites selected at the preceding higher level. The complex mathematics entailed requires a trained person to perform the calculations.

Step 11

- Record the number of sites and samples needed, for the levels that you intend to survey.

[7] This percentage value (or 'percentage point') is a function of the design prevalence percentage. For example, a confidence interval width of 5% around a design prevalence of 20% means the width is equal to 5% of 20% i.e. ± 1%. This would also mean that the confidence interval ranges between 19% to 21%.

[8] The sample size is the same for a design prevalence of 2% as 98% because the formula used to calculate sample size involves multiplying the design prevalence by 1 – the design prevalence, which means that pairs adding to 100% require the same number of sampling sites.

2.14. Step 12. Timing of the survey

Choosing when and how often to survey is another critical step.

2.14.1. When to survey

Ideally, the survey should be performed when the pest is most likely to be present and in an identifiable state.

> The timing of survey procedures may be determined by:
> - the life cycle of the pest
> - the phenology of the pest and its hosts
> - the timing of pest management programs
> - whether the pest is best detected on crops in active growth or in the harvested crop.
>
> **ISPM 6**

If the best time to survey is not known for the pest, start by finding out its seasonal habits. At what stage of the host's life cycle does the pest prefer to infest it? How long will it persist? Does it die back, such as during the wet or dry season, can it survive if the host is dead or dying? Will normal management of the crop or forest control or eradicate the pest? How quickly does it reproduce and spread? How long can the pest survive under different conditions and at different stages of its life cycle? Are there weather conditions or weather events that may influence the pest's life cycle or survival?

Other factors that may determine the timing of your survey are:

- when the pest is most active
- accessibility, and availability of vehicles
- time of local festivals or community events
- time of sowing, seedling emergence, flowering, fruit maturation and harvesting of hosts
- time of flowering for weeds
- time of obvious symptoms.

A useful website that discusses the appropriate timing for pests on a number of crops is produced by the European and Mediterranean Plant Protection Organization (EPPO). This is at <http://www.eppo.org/STANDARDS/gpp.htm>. While these are standards developed for the European climate, the information on timing is relative to the pest or host life cycle and so can be applied to other regions.

Clearly, the timing of surveying in a delimiting survey will follow the date of detection of the pest as closely as possible.

Pest lists

The timing of a survey is particularly important when developing pest lists, as it is critical that host plants are examined throughout their life cycle since different pests prefer different stages of the host development. The minimum stages of development that should be surveyed are:

- seedling emergence
- vegetative flushing stage
- flowering stage
- fruiting stage.

Examples from the case studies:

Case study C—Mahogany shoot borers: when insects are most active.

Case study E—Khapra beetle: to coincide with the peaking of beetle emergence.

Case study F—Fruit flies: continuous every 1 or 2 weeks to maintain pest-free-area status.

Case study H—Mango pulp weevil and mango seed weevil: when mango production is highest in the year.

Case study K—*Pseudomonas*: 70 days after planting to when symptoms would be visible.

Case study L—Giant wood moths: either during winter, as the exit holes are visible and new attacks are easy to assess; or during midsummer if taking specimens, as the exit holes are visible, late-instar larvae or pupae are still in the stems, and pupal skins, which aid detection, may be present.

Case Study M—Damping-off: 1 week after seed sowing, at seedling emergence when symptoms are visible.

Case Study V—Red-banded mango caterpillar: when fruit is developed but while roads remain passable.

2.14.2. Frequency of the survey

Some surveys need to be performed several times. For example, this may be every 2 weeks when managing a pest in a crop, or annually during harvest to support a pest-free-area status, or according to periods in the pest's life cycle.

If trading partners are involved, the frequency would need to be agreed upon. Also, there may be need to revise the timing and frequency if they are dependent on weather conditions or events.

The New Zealand Department of Conservation provides guidance on the frequency required in searching for weeds in forests and other natural habitats in the following publication:

Harris, S., Brown, J. and Timmins, S. 2001. Weed surveillance—how often to search? Science for conservation 175. Wellington, New Zealand, Department of Conservation.

This publication contains a table of effort required to achieve 80% and 95% certainty of detection in different habitat types and weed growth forms, and includes costs-to-control thresholds—i.e. how often you need to survey for a $500 or $5000 recovery management program.

Step 12

- Record the best timing for the survey, detailing the reasons.
- Record the frequency if the survey is to be performed more than once.

2.15. Step 13. Planning data to collect in the field

2.15.1. Identifying the sampling sites

2.15.1.1. Tagging the site

It is wise to mark sampling sites in the field whenever possible, even if you do not intend to return to the same site. It is possible that a specimen or observation taken could be lost or destroyed, and so with careful notebook entries and a marked site, you would be able to revisit the site if needed. Remember to choose tags that will survive a variety of weather conditions, and use a pencil or ink that does not smear when wetted to label the tags.

Options for marking the site include:

- spray-painting a mark
- placing sticks with a bright tassel or tag, particularly where a pest has been completely removed (such as weeds), but only when the stick or marker will not interfere with the management of the site, such as getting caught in harvesting equipment
- tying a tag or tassel to a plant stem or branch.

2.15.1.2. Recording site details

The location and unique identifying details of each site need to be recorded in a notebook. These details may be entered using a standard form that can be used for each site. For help to design your own form, see Section 2.15.2.1.

Describing the sampling site would include information such as a GPS reading, a unique number, distances from visual cues (e.g. 20 metres from roadside), number or nearest number of plant in a row (e.g. tenth tree in third row from the northeastern corner), or any distinguishing topographical features (e.g. edge of a ravine, in a ditch).

2.15.2. What data to record in the field

The most important tool you will have with you in the field will be your notebook and notes. In your notes you would record any information that could otherwise be forgotten, such as the dates of surveying, the weather at the time, the site details, the names and contact details of the local people involved, variations in who was present or absent in the survey team on the day, and any other details that you may wish to have to hand either during or after the survey.

Notebooks with carbon paper duplicate pages can be very useful when recording information to accompany a specimen taken. In this way, the details are written once only but you then have a permanent record in your notebook and a copy to be kept with the specimen. Duplicate notes could have other applications, such as at data entry time.

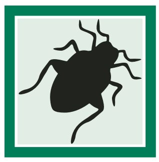
A custom-designed form is another useful tool to record data.

2.15.2.1. Designing a form

The simplest way to record data is to design a form that allows for recording all the information that you intend to collect. The forms could be bound together to ensure loose pages are not lost. Additional information that does not fit in or suit a form structure should be recorded in your notebook. You need to ensure that there is an understanding between the team members of the information to record and of a standard format, so that if multiple notebooks are used, each should be understandable at least to the team leader.

A simple way to save a lot of time is to work out ahead of the survey how the data will be stored and to design your form so that it is easy to transfer the information to the storage system. See also Section 2.17, Electronic data storage and Section 2.23, Reporting the results. When designing a form, you could include the following:

- observer's name
- field site number or name
- sampling site number or name
- targeted pest names—common and scientific
- time and date
- brief description of weather conditions
- locations, such as by GPS readings, of sampling sites
- description of habitat (e.g. aspect, vegetation, soil type)
- scale/population density categories that could be ticked
- symptoms of the pest or host
- pest life stage or state (e.g. larvae, pupae, adults for insects; anamorph/teleomorph state for fungi; seedling, budding, senescent, first flush for plants)
- caste of colonial insects surveyed, such as of termites, ants and some wasps
- behavioural notes on possible vectors (e.g. 'insect ovipositing on fruit' or 'insect resting on plant leaf')
- area or length of plot or transect assessed
- cross-reference to pest example in a pest photo library
- colour of identifying features, such as of flowers
- any quarantine measures applied at the field site, such as hygiene measures
- treatments applied to site
- additional comments.



If you are collecting specimens, you could include space on the form for:

- parasites, hyperparasites and/or biological control agents present on the specimen
- description and identifier number of specimen
- location, such as by GPS, of where specimens were collected.

See also Section 2.16.3, Labelling specimens.

Examples of information recorded in three of the case studies are:

- Case study C—Locality, situation (e.g. plantation, amenity), host species, symptoms, incidence (number of trees affected), severity (number of shoots attacked per tree), date, observer, GPS reading.
- Case study J—Cane mill area, farm name, farm number, inspection date, block number, area of block, cultivar, crop class, actual area inspected, diseases noted.
- Case study N—Location of any dead or infected tree, health status of tree, presence and extent of infection centres along a transect.

2.15.2.2. Units for data

Data are normally reported in terms of a unit of measure, usually the number of pests per unit area. The number might be a direct count of the pests or could be a scale of intensity of the pest that is recorded. The area examined might be per tree, fruit, field, crop, kilometre, quadrat, sweep of a net, trap etc. For example:

- Case study C—Number of shoots attacked per tree.
- Case study N—Number of trees affected as compared with the total number of trees examined.

In the case of surveys targeting pests that are generally expected to be absent, such as for early detection or to support pest-free-area status, the pests will rarely be found. The pest count will usually be zero, but it is still important to quantify the amount of effort expended for statistical purposes. For example, '600 trees were examined in each of 20 farms in an area, with no evidence of the pest'.

In some situations, an early detection surveillance program may regularly detect very small numbers of the pest. The total number of pests found in the region is the unit reported. An example is a fruit-fly trapping program in a border area where there are regular cross-border movements. A risk-based response strategy could be based on the number of flies trapped in a season:

- 2 or fewer—continue to monitor;
- 2–5—increase trap density;
- more than 5—incorporate quarantine and control measures to eliminate infestation.

In the case of delimiting surveys, presence or absence at a site is the essential unit of information.

Use of scales and scores

In some cases where the pest is numerous, or particularly for symptoms of plant pathogens, whole numbers of pests are not possible or useful. Instead, a scale of cover of the host or a standardised measure of the pest could be used. Scales are semi-quantitative as the scale intervals can be wide and may not be consistent in their range.

Example 1 for infection rating:

Case study M: Assigning an infection rating (area of total leaf surfaces of host affected by a pest) of zero a score of ‘0’; 1–25% as 1; 26–50% as 2 and more than 50% as 3.

Example 2 for estimating weed coverage:

The Braun-Blanquet coverage scale.

Cover class	% cover
5	75–100
4	50–75
3	25–50
2	5–25
1	1–5
Few	< 1
Rare	<< 1

Reference: Mueller-Dombois, D. and Ellenberg, H. 1974. Aims and methods of vegetation ecology. New York, John Wiley and Sons.

Example 3 for estimating crown damage in eucalypts:

This index involves visual estimates of:

- the percentage damage of entire tree crowns
- the average percent of defoliation on individual leaves
- the average percent of necrosis on individual leaves
- the average percent of discoloration on individual leaves.

The visual estimates are based on colour photographs of leaves displaying different degrees of damage.

Reference

Stone, C., Matsuki, M. and Carnegie, A. 2003. Pest and disease assessment in young eucalypt plantations: field manual for using the crown damage index. In: Parsons, M., ed., National forest inventory. Canberra, Australia, Bureau of Rural Sciences.

2.15.2.3. Importance of negative data

It is very important to record negative data, i.e. locations surveyed where the pest was not observed, so that there is a record of the effort expended to look for the pest. While this may seem obvious, it is often overlooked. It is particularly important in delimiting surveys (Chapter 5) to track pests, and in surveys to support pest-free-area status (Chapter 3).

The validity of negative records depends on a number of factors:

- the pest is known to produce easily noticed signs or symptoms
- the host species is widely distributed and has high population levels

- the host is economically important and is likely to have been examined by plant protection specialists
- the pest is relatively easy to identify
- environmental conditions are conducive to infection and pest development.

Step 13

- ▶ Decide if and how you will mark the sites. Record an example.
- ▶ Design and include a form for recording data—if appropriate.
- ▶ Do you need to collect specimens? If yes, continue to Step 14; otherwise go to Step 15.

2.16. Step 14 Methods of collecting pest specimens

It is important that pest specimens be collected and handled with the best possible care to preserve the diagnostic features for identification, especially if they are to be submitted to a permanent reference collection or herbarium.

If specimens are to be sent away for identification, often they will not be returned. Consider collecting two or more specimens, assuming you can preserve them adequately—one to keep and one to send for identification. In that way, when the specimen is identified, you will have a specimen in your possession for future reference. You may need to amend your labelling system to accommodate multiple samples.

Methods for collecting plant pests are the subject of numerous books and manuals and will not be covered here in detail. Instead, a brief review of useful references is provided below, followed by generic sampling methods for pests, to be used when specific protocols are not available. See also Box 8, What equipment to take along, on page 75.

2.16.1. Useful references

2.16.1.1. Insects and allied forms

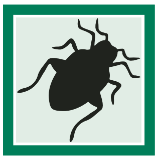
Reference one

Upton, M. 1991. Methods for collecting, preserving and studying insects and allied forms, 4th ed. Australian Entomological Society. ISBN 0 646 04569 5. This is available at <http//:www.entosupplies.com.au>. In 2005, this book was priced at $A24.20.

This small and detailed handbook covers:

- netting
- beating
- aspirating and vacuuming

- trapping
- extracting
- specialised collecting.



Reference two

Schauff, M.E. Collecting and preserving insects and mites: techniques and tools. Washington, DC, Systematic Entomology Laboratory, USDA, National Museum of Natural History, NHB 168.

This document can be downloaded from the Internet for free at: <http://www.sel.barc.usda.gov/selhome/collpres/collpres.htm>.

The book covers the equipment needed followed by information on

- trapping
- baiting, luring and other attractants
- collecting aquatic and soil insects and ectoparasites.

The book goes on to discuss killing, preservation, mounting, labelling, housing insect collections and details on packaging and shipping specimens.

2.16.1.2. Plant pathogens

Reference

Anon. 2005. Management of plant pathogen collections. Canberra, Australia, Department of Agriculture, Fisheries and Forestry.

This handbook describes the methods for collecting plant-disease specimens, covering:

- leaves, stems and fruits
- roots and soil
- macrofungi.

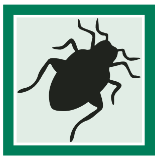
This publication would be a useful companion to these guidelines when surveying plant pathogens. It also describes how to establish a plant pathogen herbarium, as well as methods of identification and preservation of the pests to be kept for permanent collections.

2.16.1.3. Weeds

Reference

Bedford, D. and James, T. 1995. Collection, preparation & preservation of plant specimens, 2nd ed. Sydney, NSW, Australia, Royal Botanic Gardens. ISBN 0 7305 9967.

This book can be obtained directly from the Royal Botanic Gardens, Sydney. In 2005, the price was $A6.95. Go to <http://www.rbgsyd.nsw.gov.au/sydney_gardens_domain>.



2.16.2. Generic specimen collection protocols

2.16.2.1. Insects and allied forms, and plant pathogens

The generic procedures outlined here for insects and plant pathogens (see extract below) are those presented in PLANTPLAN: Australian Emergency Plant Pest Response Plan, by Plant Health Australia, 2005. For more information, go to <http://www.planthealthaustralia.com.au>.

- Sterilise any implements with a sterilant (eg. 70% v/v ethanol or 0.5% v/v available chlorine solution, as appropriate) before and after each sampling.
- If considered to be a root problem, include soil and crown (lower stem) tissues with root samples.
- It is essential that the time between sampling and dispatch of the sample for identification be kept to a minimum.
- When sampling a suspected EPP [exotic plant pest] do not drive from paddock to paddock when sampling as this increases the potential for spread of the EPP.
- If possible, sample from perceived area of minimal damage to perceived high damage within field and on individual plant

Insect samples (use specific protocols where available)

i. Where possible it is advisable to collect a large number of specimens of all life stages. For example, with the adult stage collect a number of specimens of varying size and colour depicting variation in the morphology of that species/biotype. Collection of different life stages can assist in diagnosis.

ii. Collect specimens in duplicate that are clean and in good condition, i.e. complete with appendages such as antennae, wings and legs.

iii. Use a small leak-proof alcohol resistant receptacle, such as a film canister, glass bottle with air- and liquid-tight stopper, or plastic container with screw-top lid.

iv. If sending small and/or soft bodied insects (e.g. thrips, aphids, mites and larvae), place specimen in 65% ethyl alcohol (methylated spirits can be used)–35% water and completely fill the container.

v. Tape the lid securely to avoid accidental spillage. Note: Do not remove mealy bugs or scale insects from the leaves or stems on which they are feeding as this will damage their mouth parts and make identification difficult. Instead, cut out leaf tissue around the insect and place this in alcohol.

vi. If sending hard-bodied insects (e.g. beetles, moths, grasshoppers and fruit flies), carefully fold specimen in tissue paper and place in crush-proof plastic tube or container with several holes in the lid for ventilation.

vii. Retain and store a spare sample in a secure, cool and dark location.

viii. If possible, store sample in freezer for 2 hours before dispatch to kill the insect.

ix. Clearly label all samples (see Section 2.16.3, Labelling specimens)

x. Do not send live insects.

Note: In exceptional circumstances, the diagnostic laboratory may require live material; for example, if only immature stages are available and the diagnostic lab needs to rear material through to adult (in secure facilities). In such cases, special arrangements would have to be made, ensuring secure transportation, prompt collection of samples from airports etc.

Pathogen samples (use specific protocols where available)

i. Try to select the sample on the same day it is to be sent, to ensure freshness.
ii. Select samples in duplicate. Retain second sample as reference material.
iii. For fungal and bacterial samples, store under appropriate conditions.
iv. Store sample in a refrigerator at 2–5°C until it is sent. Note: Some pathogens do not survive cold conditions. If this is true of the suspect EPP you are sampling, store under appropriate conditions.
v. Select samples at the margin between the diseased portion of the plant and the healthy portion.
vi. Select a fresh, representative and generous sample covering the full range of symptoms.
vii. If considered to be a root problem include soil and crown (lower stem) tissues with root samples.
viii. Place samples in self-sealing plastic bags with some dry tissues or paper towel to absorb excess moisture.
ix. If submitting a fruit or vegetable sample, wrap in dry tissues or paper towel and pack firmly in a crush-proof container.
x. Retain and store a spare sample using the same methods described above.
xi. Do not send dead plant material.
xii. Do not add extra moisture or pack a sample that is wet.
xiii. Do not allow sample material to dry out.

2.16.2.2. Nematodes

The extract below is from the following CABI Bioscience training manual:

Ritchie, B.J., ed. 2003. Laboratory techniques for plant health diagnostics, a practical guide for scientists, researchers and students, 11th ed. Egham, UK, CABI Bioscience.

Taking a sample

Sampling of soil that is very wet or very dry should in most cases be avoided. The soil for the sample should be taken at least 5–10cm below the surface as the nematodes congregate in the root zone. If a crop shows patches of poor growth then separate samples should be taken from the badly affected and normal areas so that a comparison can be made. Tree crops such as citrus and vines may be sampled at the drip circle[9] where the surface roots are often most abundant. Individual sample size should be about 250–300g. After the samples have been bulked and thoroughly mixed, a sub-sample of the same weight can be taken and analysed.

[9] The drip circle is where water would drip to the ground from the plant's outermost leaves.

If at all possible, roots should be either included in the sample or taken separately – about 25–100g, taken at random, should be sufficient, the lower weight being suitable for vegetables or citrus whilst the higher weight being more applicable to plants with large roots such as banana.

If stems and/or leaves appear to be attacked by nematodes, affected material can be removed and placed in polythene bags. The leaves should be removed from the bag and examined as soon as possible to avoid rotting of the tissue. Such samples should be kept separate from soil and/or root samples. Soil samples to a depth of 5 cm may be needed if above ground material is severely affected (the nematodes may be migrating to a healthy plant).

Care of samples

Samples should be placed in strong polythene bags and immediately labelled by means of a pencil-written paper or plastic label placed inside the bag.

Samples should be kept cool – do not leave in the sun or in a closed vehicle left in the sun – and should be treated with care and processed or despatched for analysis as soon as possible. If immediate despatch or processing is impossible then samples can be stored in a refrigerator at 4–8°C for several days without severe deterioration or alteration in relative composition of the nematode population.

2.16.2.3. Viruses

The following instructions are from Anon. 2005. Management of plant pathogen collections. Canberra, Australia, Department of Agriculture, Fisheries and Forestry.

Plant material that is suspected of being infected with a virus can be collected and temporarily preserved using small desiccators. This technique is best carried out at temperatures of 0 to 4°C, but will also work quite well at ambient temperatures. A plastic tube should be filled with calcium chloride ($CaCl_2$) crystals. Filling the tube up to a third of its volume is usually sufficient.

Use scissors or a safety scalpel blade to cut up leaf tissue. If the leaves are dusty or covered in sooty mould or scale insects, swabbing with water or alcohol can clean them. Leaf sections should be collected from near the centre of the lamina. Cut the leaf into 3 to 5 mm squares and place 5 to 10 squares in a plastic container containing calcium chloride ($CaCl_2$) crystals or silica gel, but separated by cotton wool … Sterilise the scissors or safety blades in alcohol or a 10% sodium hypochlorite (NaOCl) solution between samples to prevent cross contamination.

2.16.2.4. Phytoplasmas

The following instructions are adapted from Anon. 2005. Management of plant pathogen collections. Canberra, Australia, Department of Agriculture, Fisheries and Forestry.

Because phytoplasmas are obligate parasites they cannot live freely in the environment and have not been successfully grown in culture. Identification of phytoplasmas is through resultant symptoms, host range, vector specificity, appearance under transmission electron microscopy of ultra-thin sections of diseased tissue and, recently, by specific PCR primers. Specimens that are to be submitted for DNA tests could be prepared as for viral specimens. Seek advice about specimen collection and handling from your diagnostician.

2.16.2.5. Weeds

Reproduced below is a set of guidelines for collecting and submitting plant specimens, as recommended by the Australian National Herbarium. The Herbarium's website address is <http://www.anbg.gov.au/cpbr/herbarium>. At the time of writing, these details were stored at <http://www.anbg.gov.au/cpbr/herbarium/collecting/collection-procedures.html>.

Collecting

Select vigorous, typical specimens. Avoid insect-damaged plants.

Specimens should be representative of the population, but should include the range of variation of the plants. Roots, bulbs, and other underground parts should be carefully dug up, and the soil removed with care.

Make sure the specimen includes flowers and/or fruits. It may be a good idea to collect extra flowers and fruit for identification purposes.

In collecting large herbs, shrubs and trees, different types of foliage, flowers and fruits should be collected from the same plant. Collect sufficient material to fill a herbarium sheet (c. 450 × 300 mm) and still leave enough room for the label. Plants too large for a single sheet may be divided and pressed as a series of sheets.

Bark and wood samples are often desirable additions when collecting woody plants. There are special requirements for the identification of some plants. A Eucalyptus specimen, where possible, should include mature leaves, juvenile leaves, buds, fruits, and bark.

Other general hints for collecting are:

- Bulky plants or parts can often be halved or sliced before pressing. Odd fragments—bark, fruits or seeds—should be kept in numbered or labelled envelopes or packets with the main specimen.
- Very bushy twigs should be pruned to make a flatter specimen, in such a way that it is obvious where pieces have been broken off.
- With spiny plants, first place the plant under a board and stand on the board before pressing, to prevent the spines tearing the paper
- Succulent plants need to be killed first by soaking in methylated spirits for 15–20 minutes. Bulbs should also be killed, or may sprout on the herbarium sheet!
- Water plants must be floated out in a dish of water and lifted out on a sheet of stiff white paper slipped under them in the water; dry excess water, then press the plant in the usual way leaving it on the white paper on which it can remain permanently stuck. A piece of waxed paper over the top of the plant will prevent it adhering to the drying paper.
- Tall rosette plants and grasses may be pressed complete by bending them once or more into the shape of a 'V', 'N' or 'M'.
- Dioecious plants should be represented by both sexes.

- Palms—several herbarium sheets are necessary to show the various portions of the leaf, inflorescence and fruit of these species. Photographs of the tree and of each part are essential.
- Cones of some gymnosperms and Pandanaceae may need to be enclosed in a wire mesh to prevent them falling apart.

Pressing and care of specimens

Specimens should be pressed as quickly as possible after collection. If this is not possible, specimens may be stored in plastic bags, preferably wrapped in damp (but not wet) papers. Bags should not be packed tightly, and should be kept cool and moist. Make sure that each bag is correctly labelled for locality.

Place each specimen, with numbered tie-on tag attached, in a fold of several sheets of newspaper, and place in the press. If necessary, occasionally add a sheet of corrugated cardboard to act as a ventilator. As you fill the press, try to keep it level to allow even distribution of pressure. This may mean the use of alternate corners of the fold for bulky roots and other parts, or packing around a bulky specimen with foam. Close the press and exert pressure with the straps.

The plants in the press should be dried fairly quickly, in a warm place if possible. The specimens must not be left in damp papers or they will go mouldy. It is therefore necessary to go through the press daily during the first few days and change the plants into dry newspapers. Then continue to inspect press daily and change newspapers as necessary until the plants are dry.

Delicate plants and petals may be lost in changing and should be kept in tissue-paper (e.g. 'Kleenex' or toilet-paper) folders throughout changes. A properly dried plant specimen is brittle.

2.16.3. Labelling specimens

Plan to label your specimens in the field, at least in a temporary fashion until later in the day when a full and appropriate label can be made. It can be very easy to confuse unlabelled specimens, especially after some time has passed.

2.16.3.1. Minimum requirements when labelling specimens

For specimens to be scientifically useful, a set of basic data needs to be recorded at the time of collection. According to ISPMs 6 and 8, records of pest specimens collected in the field need to include as much information as possible. The list of minimum requirements varies between the two ISPMs and so both are reproduced here (facing page):

- scientific name of pest and Bayer code if available
- family/order
- scientific name of host and Bayer code if available, and plant part affected or means of collection (e.g. attractant trap, soil sample, sweep net)
- locality, e.g. location codes, addresses, coordinates
- date of collection and name of collector
- date of identification and name of identifier
- date of verification and name of verifier
- references, if any
- additional information, e.g. nature of host relationship, infestation status, growth stage of plant affected, or found only in greenhouses. Reports of pest occurrence on commodities need not be so specific on locality or verification, but should refer precisely to the exact type of commodity, the collector and the date, and if appropriate the means of collection. Reports of new occurrences of pests should also include information on any measures taken, and such reports made available on request.

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- current scientific name of the organism including, as appropriate, subspecific terms (strain, biotype, etc.)
- life stage or state
- taxonomic group
- identification method
- year, and month if known, recorded; normally the day will only be required for specific circumstances (e.g. the first detection of a particular pest, pest monitoring)
- locality, e.g. location codes, addresses, geographical coordinates; important conditions such as if under protected cultivation (e.g. greenhouses) should be indicated
- scientific name of host, as appropriate
- host damage, or circumstances of collection (e.g. trap or soil sample), as appropriate
- prevalence, indication of the level of pest presence or pest numbers
- bibliographical references, if any.

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If you intend to submit specimens to a diagnostic laboratory or expert for identification, check with them about the type and format of details that must accompany specimens. The people who will identify the specimens are likely to have advice about how the specimen should be sent in terms of its state of preservation, temperature requirements and packaging. There is more information on packaging at Section 2.16.4, General guidelines for transporting specimens.

2.16.3.2. Unique identifiers for labelling specimens

Specimens need to be assigned a unique identifier that could involve numbers, letters or a combination of both. These need to be recorded both on (or with) the specimen and in your notebook.

Develop a standard numbering system that is logical for you. If you take duplicate specimens, your numbering system should be able to accommodate this.

For example:

F23S45Sp1b: this could mean field site number 23, sampling site number 45 and duplicate b of specimen 1. In your notebook and also on the label, you would include extra information about what specimen 1 is or might be.

AW200511235a: duplicate 'a' of the 5th sample collected on 23 November 2005 by collector AW. Arranged this way, the specimen numbers will always sort chronologically. Furthermore, there is no risk of using the number again at a future date.

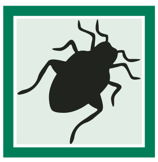
2.16.3.3. Attaching labels to specimens

Where specimens are simply wrapped in paper, the specimen details (identifier etc.) can be written on the paper, as long as it is not going to be wetted and deteriorate.

Specimen details can also be written on firm paper with pencil or permanent/waterproof markers. Make sure the writing is allowed to dry before placing it with the specimen if it is possible that the specimen will sweat or is wet. The label can be attached by tying string through a hole in the paper and securing it to a sturdy part of the plant that will not fall off if knocked or squashed.

If the specimen is in a jar or container, the jar itself is best labelled rather than the lid as once lids are removed they can easily be mixed up. Alternatively, tape a paper label or use stickers to label the container.

If specimens are to be put into alcohol in a see-through container, a paper label can be placed in the alcohol before the specimen. In this case, the label needs to be written with a moderately soft, lead pencil (HB, B or 2B) or in India ink ensuring that the ink has dried well before placing it in the alcohol. The writing needs to be visible from outside the specimen container so the label needs to be facing outwards. Do not fold the label or place two separate labels that could end up lying face to face. Small labels that float around may also damage specimens. Do not completely fill the vial with alcohol as this may allow the specimen and the label to float freely, which may increase the chance of damage to the specimen.



If pests such as insects are pinned to a surface, attach the paper label with the same pin. The label should be made of paper that is heavy enough that it remains flat and does not rotate loosely on the pin.

If you are taking soil specimens, secure labels to both the inside and outside of the specimen bag.

Microscope slides can be labelled with small stickers on the upper side of the slide, away from the specimen itself. Stickers could also be placed exactly beneath the top label on the underside of the slide, but it is important that nothing is under the specimen or it will not be possible for it to be examined under a microscope as the label will block the light.

2.16.4. General guidelines for transporting specimens

If you are transporting the specimens with you, it will be easier to ensure that they are being properly protected. If the specimens need to be sent by a shipping or postal service, greater care will need to be taken in packaging them to cope with possible mishandling during transport. Keep in mind that transport may take a few days.

If you are sending specimens to a laboratory or specialist, discuss with them how to package the specimens, when staff will be there to receive them and any other requirements they have about the preparation and transport of the specimen. Check if there are any specimen submission forms that need to be completed and despatched with the package.

Take extra care when dealing with the following:

- **Live pests.** These will require ventilation, so ensure that air can get in and the pest cannot get out. Keep plant specimens alive by wrapping in slightly damp paper and sealing in a plastic bag. Ensure that the specimens will be protected from extremes of temperature on the journey.
- **Glass or breakable containers.** These need to be packed carefully so that the glass does not touch other glass or hard surfaces and break. Such containers can be protected by packing them into a second container that is at least 2.5 cm larger on all sides, with packing material placed in the gap.
- **Multiple specimens.** If two or more specimens are to be packaged together, make sure that each is well labelled.
- **Specimens preserved in alcohol.** The containers need to be leak-proof.
- **Timing.** Submit specimens as soon as possible after collecting.
- **Postal or courier service requirements.** Check whether the postal or courier system has restrictions on sending particular volumes of alcohol, pests, container types or anything else you might think is relevant to what you are sending. This might avoid having the specimens confiscated or destroyed.

2.16.5. Special considerations when collecting a new exotic pest

As some new exotic pests pose a great threat to industry or natural environments, extreme care must be taken when a pest is first sighted or suspected to be present. If the pest has windborne spores or is a winged insect, it may be best not to disturb it as it may spread further. If a specimen needs to be collected, additional hygiene and containment steps should be taken.

It may be critical to adhere to the following instructions to ensure that a clear chain of evidence is established about the possible movements of high biosecurity risk pests, in case the pest escapes.

The instructions below refer to pests that could stick to collecting equipment, vehicles or people. For some pests, such as new fruit flies, these steps would not assist in containing the pest.

1. Leave vehicles outside the infested area.
2. Sterilise all collecting equipment before and after collecting at each site.
3. Proceed through your survey site from parts least likely to be infested to those most likely.

4. Ensure all specimens collected are well-secured and contained.
5. Do not throw away specimens that may have been cross-contaminated with a possible exotic pest. Label these specimens clearly so they can be destroyed appropriately.
6. If vehicles have been in the infested area and the pest could have adhered to the vehicle (such as seeds, pathogens in soil or fungal spores), disinfect vehicles where possible using a pressure wash with detergent within the infested area to reduce the likelihood of transporting the pest. To pressure wash vehicles in an emergency, refer to Case Study J (Section 8.11). That case study also describes personal disinfection equipment that can be carried.
7. Clothing: consider using disposable clothing such as overalls, boot covers and gloves. When you have finished at the site, place disposable clothes in a sealed bag. If the clothes can be sterilised by autoclave, use autoclave bags where possible. Use a fresh set of clothes at each survey site where the exotic pest has been found. Instead of disposable boot covers and gloves, shoe soles and hands can be sprayed with methylated spirits.
8. If the specimen is to be sent to a laboratory:
   - Pack it securely.
   - Label the package with:
     - the recipient's name, address and telephone number
     - the sender's name, address and telephone number
     - the message 'Urgent—suspect exotic plant pest specimens, keep cool'.
   - Include a covering note to the diagnostic facility outlining that the specimen is a suspect exotic pest, and indicate what you suspect the pest might be.
   - Control of the specimen must be formally passed on to each person in the chain; for example, the courier must sign for the specimen on receipt and then obtain a signature from a specified person on delivery.
   - Do not send live insects unless specifically required for identification (such as fruit fly larvae in fruit)
   - Notify the laboratory that you will be sending them a suspected exotic pest and arrange for someone to be available to collect and identify the specimen.

Step 14

- ▶ Record what types of specimens you would collect if the pest is found.
- ▶ Record how you will label the specimens.
- ▶ Record how the specimens will be prepared, treated and identified.
- ▶ Create a list of things that you will need to take when surveying.

2.17. Step 15. Electronic data storage

Irrespective of whether the data are collected in notebooks or on forms, if the numbers need to be analysed statistically or a report has to be written, the information will need to be transferred into a computer program of some sort.

You may wish to have a database program created for your survey if you have access to such resources, particularly if the survey is to be large and data entry will be repetitive.

If you think about what you will enter the data into and plan the structure of that spreadsheet or database in relation to the form and structure of notes in your notebook, it could save you and your team a lot of time and energy.

If you organise a structure before you go out into the field, it may be possible to take along a laptop computer with the program on it, so that data can be entered on-site, or after the surveying is completed for the day. A personal digital assistant (PDA) such as a Palm Pilot can also be used if available. PDAs are hand-held computers that can communicate with laptops and desktops, and are equipped with a GPS system to keep track of the user's position (latitude and longitude) to an accuracy within a few metres. They can be programmed to function as an electronic notepad form with fields to capture all the information a surveyor needs in relation to each field observation and any samples collected. The information can then be uploaded to a database in a computer on return from the survey without any need to key in the data. Otherwise, the data can be entered from paperwork when you return to your workplace.

Data need to be saved securely. Create backups of the data and keep the copies physically separate, such as on another computer or on disks or CDs and in other locations. Consider scenarios of the computer crashing and of the building catching fire or being otherwise destroyed. Ensure that the file names for backup copies are well labelled with dates or stages of the data entry that they contain and create backup copies at least weekly, or daily during the data-entry phase. Loss of even one day's data entry can be frustrating to have to repeat, and may increase labour costs.

Step 15

- Design a spreadsheet or database in which to electronically store the data.
- Decide how you will create backup copies of the data and how often you will do so.

2.18. Step 16. People

It is likely that you will have had to involve other people already if you are preparing a survey plan according to these guidelines. In step 2, Chapter 2, you will have identified experts capable of identifying the pest and laboratories where any specimens would be sent.

If your survey plan involves a statistical component, you may need to involve a statistician. You will also need to think about who will be in the survey team that goes out in the field. Consider how experienced they are in recognising the pest and if they will need training. The team will need to be informed of the whole process, including the standard methods that will be used to identify and record pests.

If you will have both men and women on your team, ensure that suitable toilet and accommodation facilities are available for everyone. You may need to consider religious, medical and dietary needs of the team members, especially if they are to be away from their home and workplace for days. You may need to ensure personal security and safety; for example, ensuring that there are always at least two people surveying in isolated situations such as forests or cargo bays in ports, and that first aid kits are available for bites, cuts and stings. Some sites may be hazardous and require special attention; for example, when heavy equipment or harvesting vehicles are present in the same area. You will need to be aware of any disabilities that a team member may have, such as poor hearing or impaired mobility, because they will need special care when around noisy or fast-moving machinery. Consider allergies of the team members and be appropriately prepared. If you will be using vehicles, be aware of the type of drivers licences that the team members have.

> Personnel involved in surveys should be adequately trained, and where appropriate audited, in sampling methods, preservation and transportation of samples for identification and record keeping associated with samples.
>
> **ISPM 6**

The size of the team may also affect morale and productivity. Having three or more members can help to keep motivation high. When members are looking for one or two pests continuously and the pests are rare or absent, consider adding additional things for the teams to look for. Keeping a record of the potential hosts (numbers and distribution) also provides positive activities that can maintain interest. Looking for and recording the status of other established pests or endangered plant species are other useful complementary activities that also maintain interest.

People's skills can be tested before going into the field, or during a pilot study. Tests of accuracy in identification can be created from photos of pests of similar appearance to the target pests or preserved specimens with the labels concealed.

Keep in mind that if the team is working long hours, and especially if the pest is mostly absent, people can become tired and bored, and their ability to observe may be lowered. If the terrain is rough or hilly, consider rotating those who will survey in the less-accessible sites. Consider planning to perform fieldwork in the mornings and processing and data entry in the afternoons, or break up the tasks in some other convenient way.

Box 8. What equipment to take along

Below is a list of equipment for you to consider taking on field trips. If you will be staying in temporary accommodation for the field trip, you may need to take extra equipment, particularly if you need to culture or preserve the samples during the trip. The list was compiled on the basis of advice from a number of people who have performed surveys.

Personal items

- Hat
- Light raincoat
- Snake-proof boots and pants
- Drinking water and food; e.g. glucose in the form of hard lollies etc.
- Mosquito repellent; fly spray can also be used on plant specimens to kill or deter insects on the specimens
- Sunscreen cream
- Sunglasses
- Bandages
- Watch
- First-aid kit: standard items plus bite cream, paracetamol, anti-diarrhoeal medication, anti-allergy tablets, antiseptic swabs, rehydrating drink sachets, chlorine water-purifying tablets, small screwdrivers, scissors, gloves and forceps
- Mobile phone with a local SIM card
- Photocopy of passport if a team member is a foreigner
- Spare clothing if surveying for quarantinable pests

Pest information sheets or pest field guide

- For more information as to what should be on pest information sheets see Section 2.4, Step 3 Identify target pests.

Recording data

- Waterproof/alcohol-proof pens/permanent markers (but not ball points) and moderately soft lead pencils (HB, B or 2B)
- Field notebook. If you use a duplicating notebook, you can record the specimen information, tear out one copy to keep with the specimen and then maintain a record of what was collected
- Waterproof paper may be needed to write on when it is raining

Specimen-collecting equipment

- Collectors tags, acid-free paper if possible
- Plastic and paper bags
- A magnifying glass/hand lens on a chain can be convenient

- Specimen tubes
- Preserving alcohol, well-sealed with a rubber top stopper (e.g. typically 70–90% ethanol)
- Fibre-free tissue
- Parafilm
- Tweezers/forceps/scalpel
- Camera
- Small pair of binoculars
- Secateurs
- Spade
- Hand-held geographic positioning system (GPS) unit that records date, time and location

SPECIAL NOTES on GPS units:

GPS units report coordinates in different ways. For example, it could be in terms of degrees, minutes and seconds or as a single number in decimal degrees. Check that any collaborative survey teams are reporting in the same unit and that the unit is acceptable to any database that the GPS reading may be entered into.

As people often fiddle with units, the coordinates can be incorrect. It is best practice to check all GPS units being used for a survey at the same spot and at the same time on each day of the survey. In this way, it will quickly become apparent if one or more have errors and need recalibrating.

- Maps
- Compass
- Diagnostic keys (identification, surveying, disease/pest rating scales)
- Collection permits, documentation of your permission to survey, permits needed to transport specimens overseas if needed
- Penknife (on a chain)
- Non-rubber household gloves/gardening gloves
- Random number generator (pack of cards, dice, calculator, stats table…)
- Cigarette lighter
- Whistle
- Disinfectant wipes (for cleaning tools to avoid cross-contamination, or cleaning hands before eating)
- Large handkerchief/hand towel
- Measuring tape
- Spray paint (for marking trees, landmarks etc.)
- Brightly coloured ribbons/tape

- Trowel or spade
- Plastic bags of various sizes—plastic zip-lock bags can be handy
- Machete
- Cardboard boxes
- Portable icebox (e.g. 'Esky' or 'chillibin')
- Small buckets (e.g. to carry intact soil samples with plants)

Survey bag

- This should preferably be waterproof and non-leather, and have a long shoulder-strap. Back-pack type bags are generally unsuitable, because it is difficult to load and to retrieve items from them. The bag should have one or two main sections with lots of little pockets.

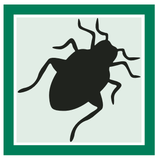
Extra items for foresters

- A hammer and chisel are invaluable for extracting small blocks of wood/bark from the stem/roots (a 25-mm chisel is a good size). Alternatively, a half axe kept sharp can also be used to extract wood/bark chips for culturing.
- A small combination pick/mattock is better than a trowel for examining the roots of trees.
- A pruning saw (ideally the folding type) is particularly useful for trimming specimens to manageable sizes.

Note: A compact version would include a sturdy knife that has a blade strong enough to prise out bits of wood/bark, a folding pruning saw, secateurs, plastic bags, permanent pens, a GPS unit, a digital camera, binoculars, a compass and a notebook.

Extra items for other plant specimens

- Water spray—use where plant specimens are to be kept alive
- Sturdy plant press. If you are visiting more than one site, use a thin or smaller one in the field and have a second one to which you transfer the specimens after surveying.
- Newspaper
- Corrugated cardboard
- Scissors, tape and clear plastic bags if using the ethanol technique (see also Section 8.21, Case study T)



Extra items for entomologists

- Sweep net
- Pooter or aspirator
- Lures or traps
- Mounting boards and pins for insects

Cottonwool to place in tube with live insect to prevent damage in field

Extra items for plant pathologists

- Spade and sieve for nematodes
- Razor blades and scalpels to section plant material for culturing
- Culture plates
- Parafilm to seal plates
- Specimen pots
- Calcium chloride chips to act as a desiccant
- Ethanol
- Ethanol flame lamp to sterilise scalpels, tweezers etc.

Wet or windy weather can be demoralising if the team is not well prepared with appropriate protective clothing, footwear, writing material and a sheltered place in which specimens can be labelled and bagged.

2.18.1. Checking for consistency in diagnostic skills of surveillance team members

To assess if people in the team similarly observe and record pests, begin by selecting five or more infested plants or fixed areas (such as for weeds) and number them. Each team member then assesses all the plants, recording details per plant on their own. Compare the results between people, both per plant and as an average over the five (or more) plants. If there are differences in the records, inspect the plants together to develop a consensus on the results. Repeat the process with new plants or sites until consistent results are obtained within the group. If there is debate concerning the diagnostic characteristics, seek further information about their appearance for the given conditions.

See also Box 6, Validating data collected by viewing from a distance, on page 48.

Step 16

- ▶ Record the members of survey team.
- ▶ Organise information and training for the team.
- ▶ Record other people who will be involved in the design, data analysis, pest identification or any other part of the survey.

2.19. Step 17. Obtaining permits and access permission

Consider whether you will need to seek permission to visit islands, villages, communities, forests or farms where you intend to survey. You will need to inform and involve people, as appropriate, particularly those in charge of the area. You will need to tell them on what dates you would like to visit and give them a clear explanation of what you will be doing and any possible ramifications for them. The timing may clash with cultural events and so access may be denied. It is possible that you will not be given access when you arrive, even if you have been given permission, because unexpected events can arise, such as a funeral procession. You may need to reconfirm permission before leaving.

You may need to obtain a visa to enter a country or island, and you may need to obtain quarantine permits for international transfer of specimens collected.

Be aware that you may not receive an immediate response and that you should check how long requests normally take to process.

You will need to allow ample time to receive these permissions and permits before your intended field trip.

Step 17

- Record what sort of permits and permissions will be needed, and who to seek them from. You may wish to note the time frames for permission to be obtained.
- Begin seeking permissions when appropriate.

2.20. Step 18. Pilot study

A pilot study involves going out to the survey site to reconnoitre—that is, to have a look at the site, or a few of the sites, to meet and inform all the people involved, to examine the conditions of accommodation and transport, and to practise surveying, collecting and transporting specimens from a site. If there is an opportunity for at least one member of the team to perform a pilot study, it can be a valuable way of increasing the quality of data obtained during the real survey. Problems encountered during the pilot study can be overcome, particularly with the help of local knowledge. You will be able to have much clearer communication with the people involved and it may be the only way to introduce yourself and your survey to the people from whom you need to seek access permission.

A pilot study can include a structured component; for example, what the expected prevalence of the pest would be. Experiments on team members' ability to detect pests could be performed in this time (see Section 2.18, Step 16 People; and Section 2.12.3.11, Drive/walk through surveys).

Step 18

- ▶ Perform pilot study.
- ▶ If you perform a pilot study, add the new information found to your survey plan.

2.21. Step 19. Performing the survey: collecting data and specimens

You and your team should now be equipped with enough plans, information and tools to carry out the survey. Good luck!

Step 19

- ▶ Perform survey.
- ▶ Collect data in the field.

2.22. Step 20. Analysing data

After your survey, you will have a set of forms or data that will be 'raw'—that is, not processed or analysed as a whole, even if all the data are zeros.

The data can be used to:

- calculate basic statistics, such as the average and total numbers of pest
- estimate the confidence of the data collected (see Section 2.13.1.4, Confidence)
- create a map of the pest distribution
- examine changes in pest locations and densities if monitored over time.

Step 20

- ▶ Store, tabulate and analyse the survey data.

2.23. Step 21. Reporting the results

As reporting requires particular attention, step 21 is covered in Chapter 7.

2.24. Where to from here?

Chapters 3, 4, 5, 6 provide extra information about detection surveys, monitoring surveys, delimiting surveys and general surveillance, respectively. Use the table of contents at the start of these guidelines to find the type of survey information you require.

> Specific surveys may be detection, delimiting or monitoring surveys. These are official surveys and should follow a plan which is approved by the NPPO.
>
> The survey plan should include:
>
> - definition of the purpose (e.g. early detection, assurances for pest free areas, information for a commodity pest list) and the specification of the phytosanitary requirements to be met
> - identification of the target pest(s)
> - identification of scope (e.g. geographical area, production system, season)
> - identification of timing (dates, frequency, duration)
> - in the case of commodity pest lists, the target commodity
> - indication of the statistical basis (e.g. level of confidence, number of samples, selection and number of sites, frequency of sampling, assumptions)
> - description of survey methodology and quality management including an explanation of
>   - sampling procedures (e.g. attractant trapping, whole plant sampling, visual inspection, sample collection and laboratory analysis); the procedure would be determined by the biology of pest and/or purpose of survey
>   - diagnostic procedures
>   - reporting procedures.
>
> **ISPM 6**

Chapter 3

More about detection surveys

Detection surveys are possibly the most common surveys performed. They involve looking for pests not known to be present. The ISPM definition is simply:

> A survey conducted in an area to determine if pests are present
>
> **ISPM 5**

As the ISPMs separate all surveys into detection, monitoring and delimiting surveys, Chapters 3, 4 and 5 have been written to align with these definitions. Both delimiting and monitoring surveys involve surveying pests that are known to be present. Therefore, any monitoring of the *absence* of a pest, is classed as a detection survey until the pest is detected.

By definition, detection surveys include surveying to develop pest and host lists (where it is not known which pests are present), as well as surveying to support pest free areas (PFAs), pest free places of production (PFPP) or pest free production sites (PFPS).

Detection surveys also include surveys of crops and forests for early detection of pests to minimise the chance of pest incursions and permit crop/forest management.

3.1. Surveying to develop pest or host lists

There are a few reasons why you might want to develop a pest or host list. Pest lists for a host or location can be used in crop management to develop a baseline of pests present in a crop or at a site. Lists are also used in pest risk analyses that form part of the negotiations in accessing international markets (see ISPM 11).

Host lists for targeted pests can provide information on alternative hosts. This can permit better pest management of crops if there are alternative host plants or sites that need to be dealt with in nearby sites or verges. The knowledge of alternative hosts can be used to determine whether a pest could pose additional risk to other crops or native vegetation in the importing country.

3.1.1. The role of pest records in pest lists

Pest lists are a compilation of any pests recorded in the area of interest. Records are usually taken from publications and reports such as NPPO records, pest surveys, research reports, journal articles and the web-based CABI Crop Protection Compendium.

Pest lists are required for pest risk analyses that are undertaken as part of negotiations for market access. Trading partners with agricultural industries at risk of exotic pests may require evidence that the pest list is accurate and reliable. Pest records will typically be the basis of the evidence provided and so can be a determining factor in whether or not market access is granted.

Pest records have a basic set of information that needs to be provided. These are set out in ISPM 8 and are reproduced in Chapter 2, at step 14 (Section 2.16). The standard provides guidance on how to assess the reliability of any pest records found. The records should be assessed in terms of the level of expertise of the collector/identifier of the pest, the techniques used to identify the pest, the conditions in which the location and date were recorded (more value is given to formal surveys than casual observations) and the publication in which the record appears (more value is placed on NPPO records and scientific journals than in unpublished documents and personal communications).

The standard also places value on specimens that have been submitted to an official[10] or general collection—a herbarium, plant pathogen herbarium or insect and allied forms collection, where a specialist will verify the identity of the pest—in addition to the recording of the details associated with the specimen.

Pest records that are linked to a specimen maintained in an official collection will provide strong evidence that the pests listed were correctly identified, as the specimens can always be viewed by others, such as trading partners, who may require confirmation.

It has been argued that pest records that do not have a specimen to verify the pest identity should be termed a 'pest report' to distinguish the quality of the observation.[11]

Pest records collected during a survey are well regarded. Certainly, pest records collected during detection, delimiting or monitoring surveys would be acceptable. However, a survey could be designed and performed specifically to increase the number of pest records for a targeted area, such as to develop a pest list for a host plant, or a host list for a targeted pest.

According to ISPM 6, NPPOs or institutions designated by an NPPO should act as a national repository for plant pest records.

Other sources of information to help identify which pests are associated with host plants are detailed in Chapter 2 at step 3 (Section 2.4).

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[10] The ISPM does not specify what deems a collection to be 'official' or 'general'.

[11] This is not to be confused with the ISPM definition of 'Pest report' applied in ISPM 17 which is used in the context of NPPOs reporting a phytosanitary barrier breach to trading partners.

3.1.2. Pest list surveys

A pest list survey involves rigorously and intensively examining the targeted hosts for any pests. Like other surveys, follow the steps described in Chapters 2 and 7.

Steps 1 to 4

Complete these steps.

Step 5

Not applicable. Alternative hosts cannot be predicted or surveyed until the pests are known.

Steps 6 and 7

Complete these steps. ISPM 4 requests additional details in the description of the area. Include the size, degree of isolation and the ecological conditions. If the targeted area is large, surveying will need to examine the range of ecological or climatic zones and all production areas.

Steps 8 and 9

Complete these steps. The districts surveyed must cover all the major growing areas for the host.

Step 10

Possible survey designs are blitz surveys (Section 2.12.3.2) and full sampling (Section 2.12.3.3), which may need to be supplemented with insect trapping (Section 2.12.3.8). These may need to be performed at a number of targeted sites.

Step 11

See Section 3.1.4, Species accumulation curves.

Step 12

Examine the plants (or among the plants if targeting weeds) at different times of the year and different stages of the host life cycle.

The timing is particularly important when developing pest lists as it is critical that host plants be examined throughout their life cycle—different pests prefer different stages of the host development. The minimum stages of development that should be surveyed are:

- seedling emergence
- vegetative flushing stage
- flowering stage
- fruiting and seeding stage.

Consider examining the host plants under different weather conditions.

Steps 13 and 14

Specimens should be collected, with details recorded in accordance with ISPM 8, and submitted to an official collection.

Examine for pests on different parts of the host plant—roots, stem, leaves, buds, flushes, fruits, seeds and any other parts—and the soil in the root zone.

Steps 15 to 17

Complete these steps.

Step 18

You may decide that you do not need to perform a pilot study especially if the survey is to be short and intense.

Step 19

Complete this step.

Step 20

As the purpose of the survey is to generate a list of pests, there is no analysis of data required, unless you wish to assess a related aspect such as a cost–benefit assessment relating time expended to the value of the data generated.

Step 21

Publication of the list in a journal or technical report is encouraged. This will not only add to the validity of the list, but also make it more widely available.

3.1.3. Example pest list case studies

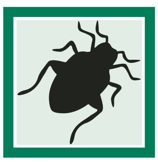
The following case studies are in Chapter 8.

Case study A

Sugarcane pests in Papua New Guinea, Indonesia and northern Australia

Case study B

NAQS and SPC early detection and pest list survey design for plant pathogens



Case study C

Pest status and early detection survey for shoot borers in mahogany and cedar trees

Case study D

Urban pest status survey in Cairns

3.1.4. Species accumulation curves (when is the list 'finished'?)

Because it may be unclear how much sampling you need to do before your pest list is 'rigorous', the concept of species accumulation curves has been developed to help make this decision. The idea is that, after looking at a number of quadrats, the number of new species added to the list will be fewer and the increasingly smaller amounts of information gained need to be weighed against the effort.

The sequential sites need to be chosen by random selection so that they are unlikely to cluster together.

If there are different parts of the field site that could affect how pests are distributed (for example, is there a fence line or creek along any of the edges, are there higher or lower parts of the ground, is there a slope?), then stratify the field site into sections of trees or area in square metres dividing up the different sections of the field site, and assign host plants or subunits of area an identifier so that locations for the survey sites can be selected.

A species accumulation curve is used to determine the number of sampling sites you need to survey. The process requires recording the number of new pests collected at each new site, then plotting the accumulated number of pest species—with sites across the X axis and number of pests along the Y axis (Figure 4). The number of new species will eventually decline with the increasing number of sites examined.

A curve of best fit is then added to the data points. When the curve has flattened for say, five consecutive sampling sites, i.e. when no or few species are added with each additional site, the survey is complete.

This exercise will probably need to be repeated in different production areas or districts if there is reason, such as different climates, to suspect that the pest list may vary from site to site.

Species accumulation curves can also be drawn for the one location but over time. This means that you would plot the number of new pests on the Y axis against time intervals on the X axis. You may wish to do this if the pest distribution on a given host is seasonal.

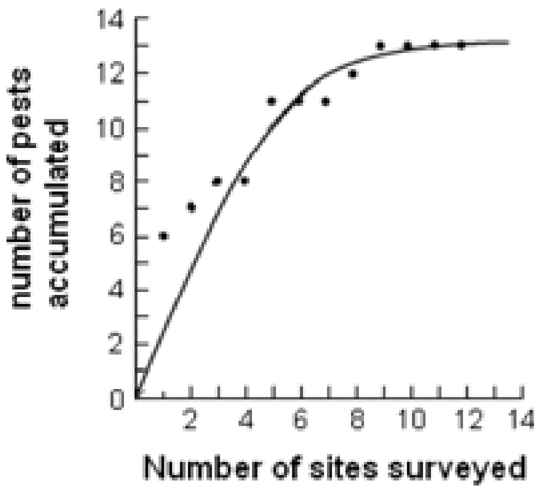


Figure 4. Species accumulation curve; returns versus sampling effort

3.1.5. Host lists and pest records

Host lists can be important to trading partners if they suspect the pest could pose a risk to a number of crops or to native vegetation as well. Host lists are also useful in pest management if multiple hosts grow near each other. In some cases, alternative hosts, such as weeds growing around fields, can present niches where a pest can survive during the months between host crops.

Pest records can be used as a basis for developing a host list for a pest. For pest lists to be useful in developing a host list, they must have information on the hosts and be searchable on this basis. Clearly, pest records that are held on searchable databases would speed up the process.

Performing a specific survey to build a host list for a pest—that is, examining many plants to determine which the pest interacts with—would be a difficult task in most circumstances. The plants surveyed could be restricted to crop plants, but this would not provide information on weedy or native alternative hosts. As a result, host lists would usually be built from general surveillance based on publications and other pest records.

3.1.6. Pest record databases

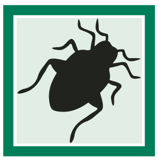
Some regional databases of pest records have been established.

- The Pacific Pest List Database developed by the SPC for the 22 Pacific Island countries and territories for their own use to facilitate trade and pest management.
- CABI Crop Protection Compendium, developed by CABI International. This can be bought online from the CABI website at <www.cabicompendium.org/cpc>.

3.1.7. Published pest lists

NPPOs are likely to have the most information on published pest lists and so you could check with them. Here are a few that are available to the public.

- Anon. 2000. List of potential plant pests already reported in Indonesia. Ministry of Agriculture, Centre for Agriculture Quarantine.
- Waterhouse, D.F. 1993. The major arthropod pests and weeds of agriculture in Southeast Asia. Canberra, Australia, ACIAR. This is provided free to developing countries.
- Waterhouse, D.F. 1997. The major invertebrate pests and weeds of agriculture and plantation forestry in the southern and western Pacific. Canberra, Australia, ACIAR. This is provided free to developing countries.



- Henty, E.C. and Pritchard, G.H. 1988. Weeds of New Guinea and their control, 4th ed. Lae, Papua New Guinea, Department of Forests, Botany Bulletin No. 7.
- Li Li-ying, Wang Ren and Waterhouse, D.F. 1997. The distribution and importance of arthropod pests and weeds of agriculture and forestry plantations in southern China. Canberra, Australia, ACIAR. This is provided free to developing countries.

3.2. Surveys to determine pest free areas, places and sites

3.2.1. Pest free area status

Pest free area (PFA) is a term that can be applied to an area of any size that is free of a pest. The term is used when negotiating and maintaining international market access.

The ISPM definition is:

> An area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained.

This statement points out that the exporting country is responsible for scientifically demonstrating that the area is free of the specific pest. Article 6 of the SPS Agreement states that any importing country has the right to ask for this evidence.

Pest free area status has the benefit that it

> … provides for the export of plants, plant products and other regulated articles… without the need for application of additional phytosanitary measures when certain requirements are met.
>
> **ISPM 4**

3.2.2. Pest free places of production and pest free production sites

If pest free status is not possible for an entire area, the status can still be established for particular places and sites within an area as alternative risk-management options for meeting phytosanitary requirements. The terms used are pest free places of production (PFPP) and pest free production sites (PFPS), where PFPS are located within a place of production.

> The concept of a pest free place of production can be applied to any premises or collection of fields operated as a single production unit. The producer applies the required measures to the entire place of production.
>
> Where a defined portion of a place of production can be managed as a separate unit within a place of production, it may be possible to maintain that site pest free. In such circumstances, the place of production is considered to contain a pest free production site.
>
> **ISPM 10**

> This standard uses the concept of 'pest freedom' to allow exporting countries to provide assurance to importing countries that plants, plant products and other regulated articles are free from a specific pest or pests and meet the phytosanitary requirements of the importing country when imported from a pest free place of production. In circumstances where a defined portion of a place of production is managed as a separate unit and can be maintained pest free, it may be regarded as a pest free production site.
>
> Where necessary, a pest free place of production or a pest free production site also includes the establishment and maintenance of an appropriate buffer zone.
>
> **ISPM 10**

Having a defined PFPP can have the additional advantage that, if it or a series of PFPPs are located within a PFA and the pest is detected, it is possible that some or all of the PFPPs can maintain their pest free status. The importing country may, however, require verification of their pest free status.

> The choice of a pest free place of production or pest free area as a management option will depend on the actual distribution of the pest concerned in the exporting country, on the characteristics of the pest and on administrative considerations. Both systems can offer adequate phytosanitary security: the main security of the pest free area lies in the common application of measures to an area covering many places of production; the main security of the pest free place of production arises from the fact that management procedures, surveys and inspections are applied specifically and intensively to it.
>
> **ISPM 10**

3.2.3. The role of surveys in determining PFAs, PFPPs and PFPSs

Surveys are only one component in the process of establishing and maintaining pest free status, as stated below:

> Three main components or stages are considered in the establishment and subsequent maintenance of a PFA:
> - systems to establish freedom
> - phytosanitary measures to maintain freedom
> - checks to verify freedom has been maintained.
>
> The methods used to achieve these components may include:
> - data assembly
> - surveys (delimiting, detection, monitoring)
> - regulatory controls
> - audit (review and evaluation)
> - documentation (reports, work plans).
>
> ISPM 4

The results of surveys will not be the sole determining factor for establishing a pest free area status. A systems approach—essentially an integrated pest management process—will be necessary (see ISPM 14). The example provided at Box 10 (page 97) of conditions imposed by an importing country on an exporting country illustrates the types of components in a system that may be required to maintain pest free area status.

In accordance with the definitions of the different types of specific surveys defined at the start of this chapter, the surveys used would primarily fall into the category of a detection survey as the pest is expected not to be present. ISPM 4 states that the surveys used in establishing pest free area status may include delimiting and monitoring surveys. These surveys become necessary when a pest is detected—a delimiting survey would be used to determine the extent of the pest incursion and monitoring surveys would then be put in place to determine changes in the pest population, such as during an eradication program. Once the pest is eradicated from the area, the survey would revert to being a detection survey. This does not mean that pest free area status will automatically return, as there may be stipulations, such as the area must be pest free for 2 years, before the pest area status can be reinstated.

Surveys can also be used to delimit the area that is free of a pest, once pest free area status has been established (see Chapters 6 and 7 for more on delimiting surveys and monitoring surveys). This section will discuss surveying only in the circumstance in which the pest is thought to be absent from the area or site.

3.2.4. Designing a survey to establish a PFA, PFPP and PFPS

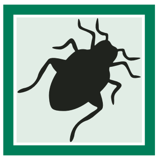
A few standards have been developed for surveillance of specific pests, but these are mostly from the North American Plant Protection Organization for its region. The standards target citrus canker, fruit flies and karnal bunt. For more information, see Box 9 below. There are also many bilateral agreements in place for pest free areas and pest free places of production. Your country may have applications that could be the basis for any new applications.

For all other surveys, the design will encompass the steps outlined in Chapters 2 and 4. Remember that the importing country will need to approve any survey protocols used, and the maintenance of a pest free area status may be audited by the importing country to verify that the pest is indeed absent.

Box 9. Pest-specific standards

Citrus canker

There is a draft ISPM for citrus canker: Guidelines for surveillance of specific pests: *Xanthomonas axonopodis* pv. *citri* (citrus canker) (2002 CEPM draft standard). This draft standard describes a specific survey plan for determining the presence or absence of citrus canker (e.g. in the establishment and maintenance of pest free areas).



Fruit flies

- Draft RSPM No. 3. Requirements for the establishment and maintenance of pest free areas for tephritid fruit flies. APPPC.[12]
- Draft RSPM No. 4. Guidelines for the confirmation of non-host status of fruit and vegetables to tephritid fruit flies. APPPC.[12]
- RSPM No. 10: Surveillance for quarantine fruit flies (in a portion of a generally infested area). This standard deals with the surveillance requirements for verifying and permanently maintaining fruit-fly-free areas within a generally infested area. NAPPO.
- RSPM No. 17: Guidelines for the establishment, maintenance and verification of fruit fly free areas in North America. This standard outlines procedures to establish, maintain and verify fruit-fly-free areas in North America. It provides measures to manage the risk of introduction and establishment of the pest, criteria for monitoring fruit flies, quarantine operations and emergency planning. NAPPO.

Karnal bunt

RSPM No. 13: Guidelines to establish, maintain and verify Karnal bunt pest free areas in North America. This standard provides guidance on the establishment, maintenance and verification of Karnal bunt PFAs and applies to seeds and grains of wheat, triticale and other hosts and regulated articles. NAPPO.

[12] At the time of publication of these guidelines, this standard had not been finalised by APPPC member countries.

3.2.5. Pest free area survey design steps

Apply this additional information to the steps outlined in Chapters 2 and 7.

Steps 1 and 2

Follow steps 1 and 2 in Chapter 2, detailing the title and reason for surveying. At step 2, include the conditions that the survey must satisfy to obtain a provisional pest free status; no pests to be detected in two life cycles of the host, for example.

Step 3

Complete this step. When applying for PFPP and PFPS, the essential attributes of the pest are:

- spread needs to be slow and over short distances
- limited chance of the pest being spread artificially
- limited host range
- low survival rate between seasons
- slow or moderate rate of reproduction
- easy to detect
- effective and practical control measures are available.

Step 4

Additional information needs to be provided about hosts, beyond what would be detailed in other surveys. In essence, the host is the commodity being considered for export. While, for example, grains contaminated with weed seeds are not a 'host' of the weed seeds, provide information about the grain plants. You will have detailed the weed seeds in the pest section at step 3.

Provide information on the location and extent of host plants within the PFA in:

- commercial production areas
- home gardens
- amenity areas
- uncultivated areas, including weed and native species, and hosts that have escaped from cultivation.

It might be useful to prepare maps showing host distribution with respect to:

- geographic features (e.g. mountain ranges, waterways)
- roads and railways
- cities and towns,
- jurisdictional boundaries
- land-use types (commercial production, residential, cultivated and public-access areas),
- individual hosts, host types and host density.

The mapped area can be divided into sub-units based on these differences; such as geography, climate, land use or accessibility.

Step 5

This step may not be applicable, but include if necessary.

Step 6

Complete this step.

Step 7

For these surveys, the area becomes the proposed PFA, PFPP or PFPS.

> A 'pest free area [PFA]' is: 'an area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained'.
>
> **ISPM 4**

You will still need to provide details as to which country and region the area is in. The borders of the area need to be clearly defined and may include administrative boundaries (e.g. country, state or province, shire or county, address), physical features (e.g. rivers, roads, mountain ranges), and geographical coordinates.

PFPP and PFPS: If a buffer zone is involved, its size should be determined by the NPPO.

Steps 8 and 9

Complete these steps. PFPP and PFPS: The acceptable characteristics of the pest free place of production or production site are as follows:

- it needs to be at a single property
- it should have clearly defined boundaries, including any buffer zones
- it should be sufficiently isolated from possible pest infestations
- no other known hosts should be within the boundaries, including the buffer zone.

Step 10

This step will not be applicable where the survey is for PFPP or PFPS, as the sites will already be determined.

For PFA status surveys, you will need to choose a method of site selection. There are few applicable site selection methods. Comprehensive data will need to be collected to allow the level of confidence to be calculated. For example, drive-by surveys would not provide data that could be statistically tested.

Appropriate methods are:

- full sampling
- random sampling
- stratified random sampling
- systematic sampling
- flying insect trapping.

Step 11

This step will be appropriate for all applications, as within PFPPs or PFPSs commodities or other parts of the commodity-handling process will need to be sampled for pest contamination.

Step 12

Complete this step. Detail how often and how long surveys must be performed to maintain the PFA status. You will need to perform the survey at intervals throughout the year or the host's life cycle. The frequency might be adjusted according to the perceived risk of the site. For example, survey low risk sites twice per year, and high risk sites at least four times per year.

PFPP and PFPS: Depending on the circumstances, the importing country may require that pest free area status be verified for 'one or more years' before the year in which export would commence, or simply from the year of export onwards.

For buffer zones:

> 'Monitoring surveys should be conducted at adequate frequency over one or more growing seasons.'
>
> **ISPM 4**

Step 13

Complete this step. PFPP and PFPS: Surveying 'may be required' of the harvested commodity at the production site.

Step 14

This step will be appropriate only to PFPPs and PFAs if specimens are to be collected when the pest is observed.

Step 15

Complete this step.

Step 16

Complete this step. PFPP and PFPS: The NPPO is responsible for the surveys, inspections and any other systems needed to verify pest status. The surveys are to be carried out by NPPO personnel, or by people authorised by the NPPO.

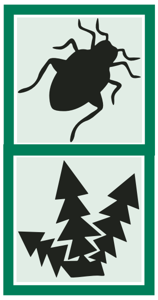
NPPO must certify the management, technical and operational skills of the producer to prevent the pest entering the place or site and their ability to manage the pest if it was detected on site.

NPPO is to provide the producer with training in pest-management systems when necessary.

NPPO is also responsible for checking the regulations of the importing country and assisting the producer in establishing conditions that would lead to compliance.

Steps 17 to 21

Complete these steps.



3.2.6. Example PFA status case studies

The following case studies are in Chapter 8.

- Case study E: PFA status survey for khapra beetle in stored grain
- Case study F: PFA status survey of Queensland fruit fly and Mediterranean fruit fly
- Case study G: PFA status survey for dodder weed
- Case study H: PFA status survey for mango pulp weevil and mango seed weevil

3.2.7. Additional steps for PFA

You will also need to detail what happens if the pest is found, and what requirements there might be before a pest free area status can be reinstated.

If eradication has been undertaken, reinstatement of pest free status cannot start until there is compliance with:

- the criteria for provisional pest free status, which may be based on the life cycle of the pest (for example, no pests have been detected for two life cycles), plus
- the pest control measures being withdrawn because they prevent the multiplication and/or detection of the pest.

3.2.8. Additional steps for PFPP and PFPS

The verification stage also requires that the commodity be labelled throughout the export process so that it can be traced back to the pest free place of production or site and traced forward to point of sale. The labelling would be critical if the pest were detected and passed through the system, as it would enable the extent of spread to be identified by a delimiting survey and increase the chance of control and eradication of the pest.

3.3. 'Early detection' surveys

Surveys designed for the early detection of new or reappearing pests in an area can use a more straightforward design than those required to obtain PFA status. The design would again follow the steps laid out in Chapters 2 and 7, except that you would work through all of the steps.

There are no considerations that are specific only to early detection surveys that are not covered in the steps presented in Chapters 2 and 7.

3.3.1. Example early detection case studies

The following case studies are in Chapter 8.

- Case study B: NAQS and SPC early detection and pest list survey design for plant pathogens
- Case study C: Pest status and early detection survey for shoot borers in mahogany and cedar trees

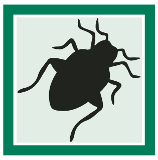
Box 10. Example of conditions imposed by an importing country on an exporting country: tomatoes from Morocco and Western Sahara to the USA

Pink tomatoes may be imported into the United States from Morocco and Western Sahara under the following conditions:[13]

- The tomatoes must be grown in the provinces of El Jadida or Safi in Morocco or in the province of Dahkla in Western Sahara in insect-proof greenhouses registered with, and inspected by, the Moroccan Ministry of Agriculture, Division of Plant Protection, Inspection, and Enforcement (DPVCTRF).
- The tomatoes may be shipped from Morocco and Western Sahara only between 1 December and 30 April inclusive.
- Beginning 2 months before the start of the shipping season and continuing through to the end of the shipping season, DPVCTRF must set and maintain Mediterranean fruit fly (Medfly) traps baited with trimedlure inside the greenhouses at a rate of 4 traps per hectare. In Morocco, traps must also be placed outside registered greenhouses within a 2 km radius at a rate of 4 traps per square kilometre. In Western Sahara, a single trap must be placed outside and immediately adjacent to each registered greenhouse. All traps in Morocco and Western Sahara must be checked every 7 days.
- DPVCTRF must maintain records of trap placement, checking of traps, and any Medfly captures, and make the records available to the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture upon request.
- Capture of a single Medfly in a registered greenhouse will immediately result in cancellation of exports from that greenhouse until the source of the infestation is determined, the Medfly infestation has been eradicated, and measures are taken to preclude any future infestation. Capture of a single Medfly within 200 m of a registered greenhouse will necessitate increasing trap density in order to determine whether there is a reproducing population in the area. Six additional traps must be placed within a radius of 200 m surrounding the trap where the Medfly was captured. Capture of two Medfly within 200 m of a registered greenhouse and within a 1-month time period will necessitate malathion bait sprays in the area every 7–10 days for 60 days to ensure eradication.
- The tomatoes must be packed within 24 hours of harvest and must be pink at the time of packing. They must be safeguarded by an insect-proof mesh screen or plastic tarpaulin while in transit to the packing house and while awaiting packing. They must be packed in insect-proof cartons or covered by insect-proof mesh or plastic tarpaulin for transit to the airport and export to the United States. These safeguards must be intact upon arrival in the United States.

[13] US 7CFR319 Subpart—Fruits and Vegetables, Sec. 319.56-2dd.

- Each shipment of tomatoes must be accompanied by a phytosanitary certificate and bearing the declaration, ‘These tomatoes were grown in registered greenhouses in El Jadida or Safi Province, Morocco, and were pink at the time of packing’ or ‘These tomatoes were grown in registered greenhouses in Dahkla Province, Western Sahara and were pink at the time of packing.’



- Case study I: Insect pests of food plants in Aboriginal communities in the Northern Territory
- Case study J: Early detection survey for sugarcane smut
- Case study K: *Pseudomonas* in rice

Consider also Case Studies L–R that were designed as monitoring surveys but could serve as early detection surveys.

3.4. References

Vernon, R. 2003. The Pacific Pest List Database for agricultural trade facilitation. Bulletin OEPP/EPPO Bulletin, 33, 501–504.

ISPM No. 4: Requirements for the establishment of pest free areas. Rome, FAO, 1996.

ISPM No. 10: Requirements for the establishment of pest free places of production and pest free production sites. Rome, FAO, 1999.

Chapter 4

More about monitoring surveys

ISPM 5 defines a monitoring survey as an

> Ongoing survey to verify the characteristics of a pest population

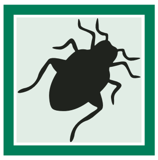
By this definition, monitoring surveys apply where a pest is known to be present and the survey is planned to examine aspects of the pest population such as the prevalence of the pest and changes in prevalence over time. These surveys can be used to assist with pest management.

The concept of being able to trade with and between areas with a low prevalence of a pest was introduced recently by the IPPC. Surveys that would underpin market access of areas of low pest prevalence fall into the category of monitoring surveys.

4.1. To support crop- and forest-pest management

The reasons you might survey a pest that is present in a crop or area are:
- to determine the optimal timing of field treatments by measuring prevalence
- to evaluate an eradication campaign at targeted sites (e.g. the perimeters of the known infestation). This overlaps with delimiting surveys (see Chapter 5). Delimiting surveys locate the boundaries of a pest infestation. Monitoring surveys can be used to determine if the pest populations change within those boundaries.

4.1.1. Pest management survey design steps



The survey design would follow all steps 1 to 21 in Chapters 2 and 7.

4.1.2. Case study examples

The following case studies of monitoring surveys are in Chapter 8.
- Case study L: Monitoring survey of giant wood moth on eucalypt and teak trees
- Case study M: Monitoring survey for damping-off in garden nurseries

- Case study N: Monitoring for root diseases in hardwood plantations
- Case study O: Monitoring survey of defoliation caused by a leaf disease in a plantation
- Case study P: Survey to measure the incidence of trees with stem wounds
- Case study Q: Monitoring survey in pine plantations
- Case study R: Aphids on crucifers
- Case study S: Monitoring survey for phosphine-resistant grain insects

4.2. To support areas of low pest prevalence status

'Areas of low pest prevalence' (ALPP) is an official term used by the IPPC in the international standards for agricultural trade. A draft ISPM on ALPPs is currently being considered—Draft ISPM May 2004: Requirements for the establishment, maintenance and verification of areas of low pest prevalence.

An ALPP is defined as:

> An area, whether all of a country, part of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific pest occurs at low levels and which is subject to effective surveillance, control or eradication measures.

They are distinguished from pest free areas as follows.

> The main difference between an ALPP and a PFA is that the presence of the pest below a specified population level is accepted in an ALPP, whereas the pest is absent from the PFA.

This means that, in some cases, a low population of the pest can be tolerated on the imported commodities, and phytosanitary measures can be employed—from seeding to selling—to manage the pests to a level acceptable to the importing country.

4.2.1. ALPP survey design steps

Apply this additional information to the steps outlined in Chapters 2 and 7. Because the draft guidelines single out extra details to be added to applications involving insects, see also Box 11 (page 102), which presents the additional specified information. Ideally, these details would already be included if the steps are followed.

Steps 1 to 6

Complete these steps.

Step 7

The NPPO should describe the proposed ALPP with supporting maps demonstrating the boundaries of the area. The description may also include the places of production, the host plants close to commercial production areas, and the natural barriers and buffer zones which may isolate the area.

Steps 8 and 9

Complete these steps.

Step 10

Sites to be surveyed should cover commercial, non-commercial and wild hosts.

Step 11

Complete this step.

Step 12

Technical reports of pest detections, phytosanitary procedures applied and results of the surveillance activities should be produced for at least the year before the application. Data should be provided for as many years as possible. One year of data may be insufficient, depending on the biology, reproductive potential, and host range of the specified pest(s).

Step 13

The NPPO where the ALPP is located should establish threshold levels for the specified pests.

Step 14

Complete this step.

Step 15

Records need to be maintained of sampling and the identification of intercepted specimens as part of the requirements of demonstrating effective phytosanitary procedures.

Steps 16 to 21

Complete these steps.

4.2.2. Additional steps for ALPP

If eradication has been undertaken, reinstatement of ALPP status cannot start until there is compliance with:

- the criteria for low pest prevalence, which may be based on the life cycle of the pest (for example, no pests have been detected for two life-cycles)
- the pest control measures being withdrawn because they prevent the multiplication and/or detection of the pest.

Box 11. Draft ISPM Appendix 1. Elements required for establishment of an ALPP for some insects

The following is a list of elements that may be considered in order to determine if an ALPP

1. Geographic description of the proposed ALPP

- maps
- places of production
- natural barriers
- buffer zone
- size
- location of regulatory control check-points.

2. Surveillance protocols for establishment and maintenance of ALPP

- pest
- surveillance time period
- reporting of surveillance results
- trapping
- trap type
- bait or lure type
- density of traps
- trap servicing intervals
- visual surveillance
- host or commodity sampling
- surveillance intervals.

3. Quality control protocols for surveillance

- validation of surveillance activities
- trapping
- visual surveillance
- verification of lure efficacy
- placement and recovery of marked pests
- regular reviews of surveillance documentation
- audits of trap placement and servicing
- confirmation of identifier competency.

4.2.3. Case study example

The excerpt below relates to an Australian import risk analysis for bananas from an area in the Philippines with a low prevalence of Moko disease.

> Bananas from the Philippines could be granted access if they were sourced from an Australian-approved plantation area, for which it can be demonstrated that the prevalence of Moko is below a level deemed acceptable by Australia—an ALPP. The low pest prevalence (LPP) level for Moko in an approved ALPP would not exceed 0.003 cases (infected mats) per hectare per week, which is about 1 case per 7 hectares per year—i.e. no more than one infected mat in 11,900 mats per year. This LPP level would be demonstrated by weekly surveys over a minimum period of 2 years immediately preceding harvest of fruit intended for export to Australia. If the prevalence of Moko exceeded the set LPP level, the affected area would be suspended for a minimum period of 2 years.

Reference

Revised draft import risk analysis (IRA). Report for the importation of bananas from the Philippines. Department of Agriculture, Fisheries and Forestry, Australia, Plant Biosecurity Policy Memorandum 2004/19, 16 June 2004.

Chapter 5

More about delimiting surveys

5.1. What is different about delimiting surveys?

The International Standard for Phytosanitary Measures (ISPM 6) defines a delimiting survey as follows:

> …survey conducted to establish the boundaries of an area considered to be infested by or free from a pest.

These surveys are usually carried out to determine the boundaries of an infestation rather than to define an area that is free from a pest.

The main difference between delimiting surveys and the other surveys covered in these guidelines is how sites are selected. The initial detection site is used as a starting point to determine how the pest arrived, where it originated and to where it might have spread. Determining to where the pest might have spread will determine where the surveying and resources for managing the pest need to be focused.

5.2. Trace-back and trace-forward techniques

Because the site where a pest is first detected might not be the initial site of the infestation, a delimiting survey can be used to identify the original source of the pest. The process of backtracking to find the original source of the pest is called 'trace-back', and the process of tracing the pest's possible spread is called 'trace-forward'. If trace-back enquiries are successful in identifying the likely original site of introduction of the pest, trace-forward activities will help to locate areas that might be infested and will need to be surveyed. The results of a delimiting survey will often have consequences for quarantine and trade and may lead to attempts to eradicate the pest. The results may be used to justify the establishment of a quarantine area around the infestation and to decide if eradication is possible.

5.3. The role of delimiting surveys in biosecurity plans

Given that the purpose of a delimiting survey is usually to identify where an exotic pest is present rather than identifying areas free from a pest, plant health authorities may have a generic survey plan for exotic pests as part of an incursion response plan. These plans are referred to as biosecurity[14] plans.

Biosecurity planning is a strategic exercise in which pest threats are identified and ranked according to the likelihood of the pests being introduced and establishing in sensitive areas where they would affect an industry. The plan would likely include strategies for preventing the entry of any exotic pests that have the potential to damage an industry, including its trading prospects. Biosecurity plans will usually identify pre-emptive actions that plant health authorities could take to reduce the impact of pests that enter and establish in a new area. These plans document how authorities would respond to a new pest incursion, such as what eradication procedures would be needed once a pest has been detected, and include instructions for performing a delimiting survey. As a result, while a delimiting survey of an exotic pest is being conducted, other processes such as eradication and sterilising of the known infested sites are often performed at the same time.

5.4. Who conducts delimiting surveys?

A delimiting survey will typically be undertaken by a regulatory agency, often the NPPO. However, the operational and regulatory capacity may reside with other agencies within the jurisdiction. In Australia, for example, plant health is the responsibility of the State governments and regulatory action on new pest incursions is usually in the hands of State agriculture departments.

At an early stage following the detection of a new pest incursion, the regulatory agency performing the delimiting survey will appoint staff to act as planning and logistics managers. They will be responsible for:

- designing and conducting the survey
- applying legislation giving authority to enter premises to undertake surveys and to apply other measures to contain the pest
- ensuring good hygiene and phytosanitary measures are applied during the survey
- record keeping.

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14 The term 'biosecurity' was brought into prominence with the introduction in New Zealand of the *Biosecurity Act* (1993) that sought to 'restate and reform the law relating to the exclusion or eradication and effective management of pests and unwanted organisms'. The term 'biosecurity' is not defined in the legislation, but a definition has been proposed by Penman (1998) as 'effective management of risks by a system of coordinated pre-border, border, management and sector responses aimed at preventing the establishment and spread of organisms that may have adverse effects on the economy, environment and people's health'.

It is essential that regulatory authorities can access the sites that need to be surveyed. In order to do this, they should have legislation in place to enable staff to enter these sites and, if necessary, to quarantine the movement of commodities, planting stock, farm machinery and any other means by which the pest might be dispersed.

The planning and logistics managers will need the support of technical specialists to identify pests that are not easily recognised or to confirm preliminary diagnosis by field staff. Technical specialists will also play a key role in advising on the biology of the pest, particularly its means of spread.

5.5. Survey design

In the absence of any biosecurity or pest incursion plans, follow the steps described in Chapters 2 and 7 with the following additional considerations and adjustments.

Steps 1 and 2

Complete these steps.

Step 3

You will need to find out as much as possible about the biology of a pest to enable you to identify all the likely sites that it might have infested. Research the epidemiology of the pest, its means of survival, reproductive rate, life span, and the effects of environmental factors.

Estimate how long the pest could have been at the site before it was detected. Even sedentary and slow-moving pests can disperse some distance if not detected early. Some pests have escaped detection for several years.

You will need to closely consider how the pest can be spread, as discussed in Box 12 on the next page.

Steps 4 and 5

In completing these steps, you will need to identify all the known hosts and where these are, especially those that are close to the site of detection. A list of properties and sites with host plants can be developed from a number of sources, including industry and government records and personnel, local grower groups and cooperatives, fruit packers and distributors, extension staff, researchers and property owners. If available, aerial photographs can also be useful for identifying areas that are densely populated by hosts, such as production areas. For cultivated hosts, the most susceptible varieties are targeted if these are known.

Consider all alternative hosts, as well as the susceptibility of endemic flora in remnant forest, parklands, gardens and other areas close to the detection site. Keep in mind that the symptoms might be masked or subtle on resistant hosts. Viruses might be latent in some cultivars with the host expressing symptoms only in response to particular environmental conditions, or when in mixed infections.

Step 6

Complete this step. Access any biosecurity or pest incursion plans as a priority.

Box 12. Human-assisted and natural dispersal of pests

Human-assisted dispersal

A priority for survey managers will be to determine the likelihood that the pest may have been dispersed with commodities from packing houses and planting stock from commercial nurseries or seed suppliers. These commodities have the potential to disperse an exotic pest over great distances and to many sites very quickly. The immediate destinations of bulk commodities and planting stock will likely be documented, but sales from retailers may not be so readily traced.

Consider also the following:

- Is the pest likely to have been carried by workers and their equipment to other properties, including their homes?
- Is harvesting equipment shared between properties?
- Are packing boxes and other storage materials re-used?
- What vehicles could have come in contact with the pest and where have these travelled?
- How are agricultural wastes, such as manure, disposed of?
- Has infected produce or plant material entered the market?

Natural dispersal

Consider the following:

- Is the pest spread by wind or rain?
- What is the prevailing wind direction and what have been recent weather conditions in the area?
- Can the pest be dispersed by waterways, irrigation channels or by flood events?
- Over what distance is the pest likely to spread by natural means considering recent weather conditions, life stage of the pest, flight and survival characteristics?
- Is the pest vectored by insects, mites, fungi, nematodes or other organisms?
- Are vectors present in the area and, if so, at what density?
- How effective are vectors at transmitting the pest? Does the pest replicate within the vector?
- Do endemic close relatives of known vectors also transmit the pest?
- Is there dropped fruit on the ground that could harbour the pest?
- For weed seeds and the seeds of parasitic plants, are they dispersed by birds?
- Are there geographical features, such as a sea border, that limit the direction in which a pest can travel?

Steps 7 to 9

Determining the sites will be largely based on the nature and dispersal of the pest and the nature and distribution of hosts (steps 3 to 5). The sites would need to include distribution points of infested host material, such as produce or root stocks that may have entered the market.

You may need to develop a questionnaire. Read Box 13, Using a questionnaire to identify survey sites (page 108), which includes information on designing a questionnaire.

As a result, the survey may need to include all production places within an area, district or place and to target all susceptible species in orchards, nurseries, areas of natural vegetation, residential, public and commercial properties.

Step 10

The survey design is simply a delimiting survey as detailed in these steps.

Step 11

While statistics will not be needed to calculate how many sampling points are required, you may have to choose a sampling structure, such as a grid of traps, that is statistically sound. A random element could be added by inspecting a few sites where it is thought that the pest is unlikely to be present or that is between sampling points, such as weeds on road verges.

Step 12

Not applicable as the timing will be set by the date that the first detection was observed and how quickly a survey can be planned and organised.

Steps 13 and 14

Specimens should be collected together with details in accordance with ISPM 8, and submitted to an official collection to confirm identification and to provide a pest record. See also Chapter 3.

Steps 15 and 16

Complete these steps.

Step 17

While a NPPO may have legislation in place to access sites, you may still need to approach people to gain access to sites such as domestic gardens or farms.

Step 18

It is unlikely that there would be sufficient time to perform a pilot survey, unless the plan is designed and practised before the target pest is actually detected.

Step 19

Complete your survey.

Box 13. Using questionnaires to identify survey sites

An important component of the delimiting survey is a trace-back and trace-forward analysis to determine the source of the outbreak and to identify other premises that might have been exposed because of closeness to or contact with the infested property, through movement of infected planting material or fruit, or by sharing employees and equipment.

Face-to-face interviews or questionnaires distributed to property owners can be a useful way of determining whether hosts are present on a property. The questionnaire will be useful for determining production details, the ownership of properties where hosts occur, and for collecting preliminary trace-forward and trace-back information, including the source of planting material, movements of equipment and staff, and propagation practices. This information will allow you to develop a risk profile for each property.

See also step 4 in Chapter 2.

Questionnaires are particularly useful in trace-forward and trace-back investigations where seed or other forms of planting material are suspected as the source of the pest, especially if you can verify any reported instances of the pest. A questionnaire may be of less use in trace-forward and trace-back investigations when the pest has blown or flown in.

Where the new pest is associated with purchased seed or planting material it will be necessary to go to the supplier and repeat the survey questionnaire to seek out the original source of the planting materials and to identify the places to which the planting material, and therefore the pest, might have been distributed. The same set of questions should be put to farmers, seed suppliers and nursery owners who can be traced from answers to the questionnaire.

Designing a questionnaire

The questions could be written to identify:

- the source of the planting material
- the destination of plants and plant products which may have been moved from the property, be it a farm, an orchard or a nursery
- the location of properties that share equipment or have labour that moves from property to property such as when harvesting fruits and vegetables
- adjacent fields/properties owned by the farmer on whose land the pest was detected
- the movement of commercial apiarists (if applicable)
- the movement of other visitors that may have been on the property
- climatic conditions or weather events that might favour the establishment and spread of the pest.

- Enquire about people who may have been travelling, especially internationally, as they may have brought pests back with them. There are reports based on circumstantial evidence that some rusts, smuts and ergots might have been introduced into new areas on the clothing of returning travellers.

If the responses to the questionnaire are to be entered into a database or other computer program, a simple way of saving a lot of time is to design your questionnaire and database so that they match in design layout. This will speed up the process of entering the data into the storage system. See Step 15, Chapter 2.

Once results are received from questionnaires and interviews with workers, property owners etc. these are used identify other sites and locations that must be surveyed.

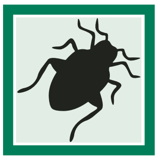
Step 20

As the purpose of the survey is to identify where the pest has spread, a map of its distribution would be useful.

Step 21

Complete this step.

5.6. Example delimiting survey case studies



The following case studies are in Chapter 8.

- Case study T: Delimiting survey of papaya ringspot virus
- Case study U: Delimiting survey for Huanglongbing disease of citrus and its vector the Asian citrus psyllid in Papua New Guinea
- Case study V: Delimiting survey for red-banded mango caterpillar in northern Queensland
- Case study W: Delimiting survey of the Queensland fruit fly in Rarotonga, Cook Islands.

Reference

Penman, D.R. 1998. Managing a leaky border: towards a biosecurity research strategy. Wellington, New Zealand, Ministry of Research, Science and Technology, 61p.

Chapter 6

More about general surveillance

ISPM 6 briefly covers what is *required* under the term ‘general surveillance’. The requirements fall into two categories of activities. The first is collecting information about a pest. The second is to develop clear communication between NPPOs and other people who have information about pests.

6.1. Collecting information about a pest

ISPM 6 refers first to the process of collecting information about a pest. This is covered in detail at step 3 in Chapter 2 (see Section 2.4.1). The standard then requires that the information collected from the various sources be compiled and verified. The information should be both stored and retrievable. Verifying the information sources on pests is also covered under step 3 in Chapter 2 (see Section 2.4.2).

The ISPM states that this information about the pest can be used to:

- support NPPO declarations of pest freedom
- aid early detection of new pests
- report to other organisations such as RPPOs and FAO
- compile host and commodity pest lists and distribution records.

In other words, the information collated can be used as one part of designing specific surveys as described in Chapter 2, or it may be adequate on its own to develop a report of the pest status of an area which can be used for other purposes. If the information compiled is inadequate for these other purposes, then specific surveys can be performed to provide extra information about the pest.

This process of compiling information on pests is also necessary when developing target pest lists. See Box 14, Developing target pest lists, on the next page.

Box 14. Developing target pest lists

Pest lists are an inventory of pests in an area. Target pest lists are an inventory of pests in the surrounding regions and countries that threaten to enter the area.

Target pest lists are used to focus surveillance activities and incursion management planning on high-priority threats. Depending on their purpose, target lists vary in their scope. For example, they could include all high-priority exotic pests that threaten an industry on all pathways from all sources, or simply focus on pests of concern from one source along one pathway.

The development of target lists is based on identifying pests that could arrive, then performing a risk assessment for each pest.

If the list of pests is developed as part of a market-access application, the list is restricted to the range of pests associated with commodity host plants and materials in the exporting country or region. If the list is being developed to create a list of quarantine pests, then all pests from all neighbouring countries need to be included, as well as from countries from which people and cargo arrive.

To identify pests associated with a host or a neighbouring area, a pest list needs to be developed. This process is detailed in Section 3.1.

Assessing the threat posed by the listed pests

The aim is to assign an overall level of risk for each pest, based on the probability of its entry, establishment, spread and consequences. The overall risk rating is usually expressed in qualitative terms (e.g. on a scale of 1 to 5, or in terms of 'low', 'moderate' or 'high') and can be used to decide which pests pose a higher priority and warrant inclusion on a target list.

Guidance on the process and considerations involved in assessing pest risk are provided in detail in ISPM 11, Pest risk analysis for quarantine pests.

6.2. Open communication channels with NPPOs

The standard requires that there be *communication channels to transfer information from the sources* [of pest information] *to the NPPO*. It proposes that, if necessary, the communication could be improved by introducing incentives to people to report information about a pest. The incentives suggested are:

- legislative obligations (for the general public or specific agencies)
- cooperative agreements (between the NPPO and specific agencies)
- use of contact personnel to enhance communication channels to and from NPPOs
- public education/awareness programs.

There are no further details about these suggestions in the ISPMs. The next section contains information about public education/awareness programs.

Another incentive that has been used successfully[15] is to provide a free pest identification service, which encourages people to become involved and to send in unusual specimens.

6.3. Developing awareness campaigns

Awareness campaigns are often initiated to alert farmers and the public to the detection of a pest new to an area, the likely arrival of an exotic pest or when an endemic pest increases in significance in response to changing environmental conditions or cropping practices. The awareness material has two main thrusts: to inform the audience about the targeted pests and to provide the audience with instructions on how they can assist.

6.3.1. Providing information about pests

Providing information on the pest can be achieved using a range of media. These fall into the categories of handouts and public announcements.

6.3.1.1. Preparing handouts

Raising awareness through the preparation and distribution of booklets or fact sheets (sometimes called 'pest alerts') is a common approach. Postcards, posters, calendars and bookmarks are other easily distributed materials. The information could be included in existing newsletters.

Effective material allows the audience to readily recognise the pest or pest symptoms. Therefore handouts should include:

- information about the name of the pest and why it is important
- descriptions of the pest and/or pest symptoms
- colour photographs of the pest and/or symptoms
- description of the host or environment where the pest might be found
- when the pest might be found, for example, in terms of seasons or host growth stage
- who you are—the group preparing the handout and why it is your topic.

Other attributes of the handout should be that:

- the material is easy to read and understand
- the material captures the reader's attention
- the information is presented on a single sheet of paper—single or double-sided.

If you make computer files of the handouts available (such as on the Internet), remember that the handouts are most likely to be printed out in black and white, which may affect the information that you have provided. Print it out and look at what other people would see and make any improvements possible.

[15] By NAQS, as part of an integrated system to protect Australia's northern borders from exotic pests

6.3.1.2. Pest alerts

Pest alerts can be structured as follows: introduction (circumstances in which the pest has been found), identification of the pest, biology of the pest, distribution and hosts, symptoms on hosts, further reading, and how to report a sighting of the pest. There are many pest alert examples available on the Internet.

Example: SPC

Pest alerts released by the SPC can be viewed at <http://www.spc.int/pps/pest_alerts.htm>.

6.3.1.3. Booklets

Small flip charts can be made that describe a series of pests that people can look out for. Typically, these are small, sturdy, ring-bound booklets with water-resistant pages. They are small enough (e.g. page size 11 by 15 cm) to fit easily into a car glove box or to be carried. The pages on each pest would have colour photos of the pest and/or symptoms, then brief details of the pest's name, characteristics, hosts, known distribution, potential impact and any other useful information such as other organisms it could be confused with.

Example: WEEDeck

The WEEDeck series targeted selected weeds exotic to Australia; see <http://www.weeds.org.au/weedeck.htm>, with examples of weed cards provided on the publisher's website at <http://www.sainty.com.au/weedeckpg1/weedeckpg1.html>.

Example: Forests and timber: a field guide to exotic pests and diseases

This booklet of Australian forest pests is available free from the Australian quarantine website at< http://www.aqis.gov.au> by selecting 'Publications' then choosing 'Timber—a field guide to exotic pests and diseases'.

6.3.1.4. Public announcements

Awareness activities may also include public seminars, talks with small groups of locals, information stalls at community events, road signage, and radio and television announcements or press releases. If your organisation has a website, information—including copies of pamphlets and pest alerts—can be made available to anyone with access to the Internet.

The timing of campaigns can be important to the success of the campaign, as demonstrated in the example on the next page, describing involvement of the public in Siam weed (*Chromolaena odorata*) eradication in Queensland, Australia.

Example: 'Lord of the weeds' school competition

The Cooperative Research Centre (CRC) for Weed Management has run a competition in schools in which students design a strategy to manage weeds that are in the school, or in the local area. The winning school was awarded $1000 to be used as they wished. The CRC provided suggested lesson activities, student proformas to guide students through writing the report and, for teachers, a marking guide and appropriate contacts. These materials and further information are available on the Internet at <http://www.weeds.crc.org.au/education_training/school_resources.html>.

Example: Involving the public in an eradication campaign for Siam weed

The Northern Australia Quarantine Strategy (NAQS) undertakes vigorous public-awareness campaigns to bring potential pests of quarantine concern to the attention of the general public. This includes talks and demonstrations to schoolchildren, landholders and interest groups (e.g. Landcare groups); media articles and radio broadcasts (in regional areas); preparation and distribution of awareness material including newsletters, booklets and calendars; and inviting landholders and government officials to submit specimens of unknown pests or weeds for identification.

Effective public-awareness campaigns can assist in mapping the distribution of weeds. As part of a campaign to eradicate Siam weed, the Queensland Department of Natural Resources and Mines undertook a vigorous advertising campaign timed to coincide with this species' flowering season in May to August, when it is usually most conspicuous. Television advertising and newspaper articles showed the plant in flower and advised concerned residents who to contact if they thought they had seen the weed. This publicity campaign led to the reporting and confirmation of four previously unknown infestations. Strategic advertising for this and other species that are eradication targets will continue to play an important role in the success of the eradication efforts.

Television and newspaper advertising timed to coincide with the flowering season of a weed can be valuable in identifying new infestations. While television is probably the most effective tool, the charges for production and advertising are likely to be prohibitive and thus may not be the most affordable means. In the case of the Siam weed eradication campaign in Queensland, the cost was reduced by television stations agreeing to broadcast the advertisements at little or no cost as part of community service obligations. Posters, photographs, talks accompanied by slide presentations, live specimens (where legislation permits such use) and laminated herbarium specimens also provide useful tools to illustrate weeds of concern to the general public. In far north Queensland, NAQS illustrates several weeds of concern in the annual Torres Strait and Cape York calendars, with photographs appearing on the month when that species is most likely to be flowering. In all cases, it is vital that members of the public be informed about who they should contact if they think they have found a weed of concern. Timely identification and feedback is provided for all reports made or specimens submitted.

6.3.2. Targeting audiences

People who work regularly among host crops or in targeted areas are likely to be aware of the pests that are normally present and so be most likely to notice a new pest or anything unusual. Such groups of people include farmers and farm personnel, extension staff, field technicians and interested community groups. The general public can also be very helpful in increasing the area covered and the number of people looking for the pest, as can experts in taxonomy and plant health. Projects could be set up to involve students and staff in schools and universities to both increase their knowledge of entomology or plant pathology and to help with finding pests. Domestic pest controllers and staff at garden nurseries can also be useful groups to assist with reporting new insect pests.

It is important to identify and inform any groups who may already be conducting pest surveys or control programs of pest issues, as they may be unaware of other pest surveillance programs operating in their region.

Example: Forests and timber: a field guide to exotic pests and diseases

This booklet (see page 114) targets people who work with timber—wharf workers, container depot staff, timber handlers, timber-yard workers, forest workers and forest technical staff.

6.3.3. Reporting networks—how the audience can report pests

Once you have informed the people who may be able to assist in detecting a pest, you need to have a way for people to let you know, and a system in place to keep track of pest reports. This will enable you to manage a series of pests and provide you with the information to assess if the campaign is effective.

Some of the options that have been used are free-call telephone services, text messaging to a central database, and providing a direct contact number, fax and email of a plant protection officer on handouts.

Example: Pestex early warning system for pests of corn

The Philippines Department of Agriculture has developed a corn (maize) pest surveillance system called Pestex to help prevent pest outbreaks and reduce economic losses caused by plant pests. One of the objectives of the program is to establish a farmer-based surveillance network in order to determine pest status, generate forecasting data and provide information to assist pest-management decisions. Farmers and agricultural technicians report pest data to a central authority (the Bureau of Primary Industries) by mobile phone text message. Information is added to a database and verified by technicians who either visit the areas reported to be affected, or seek samples from more remote areas. The appropriate response plan is then implemented.

Example: Telephone hotline for pest reporting

The exotic plant pest hotline is a freecall telephone service provided primarily for members of Australia's plant production sectors and plant health services to report suspect exotic plant pests. The hotline is staffed during business hours. Callers are directed to government staff in their State who have expertise on the pest and who are able to determine what course of action should be taken.

The hotline number is promoted by the Australian Government Department of Agriculture, Fisheries and Forestry's public relations section, through awareness campaigns that distribute the number on pamphlets and bookmarks, and by including the number on all pest booklets produced.

For more information go to <http://www.outbreak.gov.au>.

Example: GrainGuard

In Western Australia, the Department of Agriculture operates GrainGuard, a program targeting both specific and general surveillance of grain threats. The program involves growers and agribusiness, integrating grain pest response activities in Western Australia. It includes distribution of information on exotic pest threats of grain crops and collection kits to promote submission of suspect exotic pests to the Department of Agriculture. For more information go to <http://www.agric.wa.gov.au>. Choose 'Crops' on the menu then 'GrainGuard'.

Chapter 7

Step 21. Reporting the results

7.1. To whom should you report?

If you were funded to do a survey, it is likely that your funding body will require a report of the survey. If the survey was designed for trade-related purposes, NPPOs will need to be given a copy of the report. If you are representing the NPPO, there are obligations as to whom notifications of trade-related pest detections must be reported. More information is given at Sections 7.7 and 7.8. If you belong to an academic institution, you may need to produce a report for your head of department, or you may intend to submit the findings to a journal.

7.2. Writing a summary

It can be useful to have a simple summary that can be provided as follow-up information for the people who were involved in the survey; from the team members to the local farmers, rangers and community leaders. This will acknowledge their assistance and show that their involvement was appreciated. This is particularly important if you need to continue to return to the sites, such as for monitoring of pests, because you need to keep the communication channels open with everyone involved.

Reports or summaries that are to be given to people involved in the survey can be much simpler than a full report and may be reduced to a handout or pamphlet. In this instance, less detail is required, but content such as photos and anecdotes are encouraged.

In this situation, the pamphlet text might include:

- the survey title and team members
- the aim of the survey, including which pests, hosts and sites were targeted and why
- what was found
- what it means to the people who will read the pamphlet

More information is provided about pamphlets and educational material in Chapter 6.

7.3. Press releases

A summary may be sufficient for press releases. If you need to write a press release, you will be working for an organisation that will likely have public relations staff who can help you with the structure and appropriate content of the release, and with its distribution. Some organisations, such as the SPC, include press releases on their websites; go to <http://www.spc.org.nc/> and choose 'Press releases' from the menu.

7.4. Newsletter articles

Newsletters are typically a way of informing a select group, such as fruit growers, as to what is current news in the field. Depending on the newsletter, a simple summary and contact details may be sufficient. Others may want submissions that are more detailed and more like a journal article.

7.5. Writing a basic report

A basic report would include material from a number of the steps in the survey plan, so most of the work is done already, and the writing will be a shortened version of this accompanied by results and an interpretation of the survey findings.

7.5.1. Components of a basic report

A basic report should provide at least the following information:

- the survey title and team members, from step 1
- the reason for surveying, from step 2
- background information on the pest, host and sites of interest, including discussion of any earlier, related surveys, from steps 3–6
- the survey design methods in detail—this would include site selection from steps 7 to 11, timing of the survey from step 12, the type of data and specimens collected, from steps 13 and 14
- how the data were analysed and interpreted, from step 20
- conclusions that can be drawn about the survey findings, and how these relate back to the purpose of surveying.

The report could also have a brief abstract near the beginning and could include a glossary of terms and acknowledgments such as from whom permission and funding were received.

7.6. Formal reports with set formatting

For reports that are to be submitted to funding bodies, NPPOs or journals, there will be information provided by the organisations as to how the report should be written and formatted. This information needs to be sought from the specific organisation.

For situations involving trading partners, there can be obligations as to the format and content of pest reports. These are covered in ISPMs 13 and 17. The reporting obligations are reproduced in part in Sections 7.7 and 7.8.

7.7. ISPM 13—Reporting of pests in imported consignments

This standard describes as follows the actions to be taken by NPPOs about the notification of:

- failure to comply with phytosanitary requirements
- detection of regulated pests
- failure to comply with documentary requirements, including:
  - absence of phytosanitary certificates
  - uncertified alterations or erasures to phytosanitary certificates
  - serious deficiencies in information on phytosanitary certificates
  - fraudulent phytosanitary certificates
- prohibited consignments
- prohibited articles in consignments (e.g. soil)
- evidence of failure of specified treatments
- repeated instances of prohibited articles in small, non-commercial quantities carried by passengers or sent by mail…
- an emergency action taken on the detection in an imported consignment of a regulated pest not listed as being associated with the commodity from the exporting country
- an emergency action taken on the detection in an imported consignment of organisms posing a potential phytosanitary threat.

The importing contracting party is required to notify the exporting contracting party as soon as possible about significant instances of non-compliance and emergency actions applied to imported consignments. The notification should identify the nature of non-compliance in such a way that the exporting contracting party may investigate and make the necessary corrections.

Notification should be timely and follow a consistent format. Where there is a significant delay in confirming the reason for the notification (e.g. identification of an organism), a preliminary notification may be provided.

7.7.1. Format of notifications

Notifications should include the following information:
- *Reference number*—the reporting country should have a means of tracing the communication sent to an exporting country. This could be a unique reference number or the number of the phytosanitary certificate associated with the consignment
- *Date*—the date on which notification is sent should be noted
- *Identity of the NPPO* of the importing country
- *Identity of the NPPO* of the exporting country
- *Identity of consignment*—consignments should be identified by the phytosanitary certificate number if appropriate or by references to other documentation and including commodity class and scientific name (at least plant genus) for plants or plant products
- *Identity of consignee and consignor*
- *Date of first action* on the consignment
- *Specific information regarding the nature of the non-compliance and emergency action* including:
  - identity of pest
  - whether part or all of the consignment is affected
  - problems with documentation
  - phytosanitary requirements to which the non-compliance applies
- *Phytosanitary actions taken*—the phytosanitary actions should be specifically described and the parts of the consignment affected by the actions identified
- *Authentication marks*—the notifying authority should have a means for authenticating valid notifications (e.g. stamp, seal, letterhead, authorised signature).

  For more information, refer to ISPM 13.

7.8. ISPM 17—Pest reporting

This standard describes the responsibilities of and requirements for contracting parties in reporting the occurrence, outbreak and spread of pests in areas for which they are responsible.

It also provides guidance on reporting successful eradication of pests and establishment of pest free areas. These reports are termed 'pest reports'.

7.8.1. Content of reports

A pest report should clearly indicate:
- the identity of the pest with scientific name (where possible, to the species level, and below species level, if known and relevant)
- the date of the report
- host(s) or articles concerned (as appropriate)
- the status of the pest under ISPM 8

- the geographical distribution of the pest (including a map, if appropriate)—the nature of the immediate or potential danger, or other reason for reporting. It may also indicate the phytosanitary measures applied or required, their purpose, and any other information as indicated for pest records in ISPM 8 (Determination of pest status in an area).

If all the required information is not available on the pest situation then a preliminary report should be made with updates as further information becomes available.

7.8.2. How to submit reports

Pest reports which are obligations under the IPPC should be made by NPPOs using at least one of the following three systems:

- direct communication to official contact points (mail, facsimile, or email)—countries are encouraged to use electronic means of pest reporting to facilitate wide and prompt distribution of information
- publication on an openly available, official national website (such a website may be designated as part of an official contact point)—precise information on the website access address to the pest reports should be made available to other countries, or at least to the Secretariat of the IPPC
- the International Phytosanitary Portal.

In addition, for pests of known and immediate danger to other countries, direct communication to those countries, by mail or email, is recommended. Countries may also address pest reports to RPPOs, to privately contracted reporting systems, through bilaterally agreed reporting systems, or in any other manner acceptable to the countries involved. Whatever reporting system is used, the NPPO should retain responsibility for the reports.

Publication of pest reports in a scientific journal, or in an official journal or gazette that typically has limited distribution, does not meet the requirements of this standard.

7.8.3. Timing of reporting

Reports on occurrence, outbreak and spread should be provided without undue delay. This is especially important when the risk of immediate spread is high. It is recognised that the operation of the national systems for surveillance and reporting, and in particular the processes of verification and analysis, requires a certain time, but this should be kept to a minimum.

Reports should be updated, as new and more complete information becomes available.

For more information, see ISPM 17.

Chapter 8

Case studies

8.1. Case study attributes

Case study	Survey type	Pest type	Host common name	Vegetation	Country	Site selection method
A	Pest list	Plant pathogen	Commercial and wild sugarcane cultivars	Community gardens, domestic gardens and road verges	Papua New Guinea, Indonesia, northern Australia	Targeted
B	Early detection, pest lists	Plant pathogen	Range including: bananas, citrus trees, sugarcane	Urban, agroforestry, orchard and fields	Pacific islands, northern Australia, Torres Strait, Papua New Guinea, Indonesia	Targeted, convenience
C	Pest status, early detection	Insect	Mahogany and cedar trees	Plantations and amenity trees	Fiji, Vanuatu, Tonga, Samoa	Targeted, drive-by
D	Pest status	Plant pathogen	Target list included mango, citrus, bananas, cucurbits, grapes and other Vitaceae and Malvaceae species, solanaceous crops	Urban, domestic gardens, high-risk sites, public parks, feral commercial plants	Northern Australia	Targeted
E	Area freedom	Insect	Stored grain, including wheat, barley, oats, rye, maize and rice	Commodity	Western Australia	Targeted, trapping
F	Area freedom	Insect	Apple, pear, apricot, nectarine, peach, citrus	Orchard	Southern Australia	Systematic trapping
G	Area freedom	Weed	Amongst Niger seed, sorghum, pearl millet	Field	Northern Australia	Targeted, convenience
H	Area freedom	Insect	Mango	Orchard and urban	Guimaras Islands, Philippines	Randomised

Case study	Survey type	Pest type	Host common name	Vegetation	Country	Site selection method
I	Early detection	Insect	Thirteen target list food plant groups	Domestic gardens	Northern Australia	Targeted, convenience
J	Early detection	Plant pathogen	Sugarcane	Field	Northern Australia	Targeted, randomised
K	Early detection	Plant pathogen	Rice	Field	Thailand	Systematic, transects
L	Monitoring	Insect	Rose gum, Dunn's white gum, forest red gum, river red gum	Plantations	Southern Australia	Stratified, transects
M	Monitoring	Plant pathogen	Any seedlings	Garden nurseries and greenhouses	Any	Targeted, full sampling
N	Monitoring	Plant pathogen	Hardwood plantations including hoop pine	Plantations	Any	Targeted
O	Monitoring	Plant pathogen	Gum tree	Plantations	Southern Australia	Stratified
P	Monitoring	Plant pathogen and insects	Shining gum	Natural forest	Southern Australia	Targeted, randomised
Q	Monitoring, pest status	Plant pathogen and insects	Pine tree	Plantations	Southern Australia	Vantage point
R	Monitoring, pest status	Insect	Crucifers including cabbage, brussels sprouts, radish, cauliflower, tobacco	Field	Vietnam	Convenience, systematic
S	Monitoring	Insect	Stored grain including wheat, barley, oats, rye, maize, rice	Commodity	Western Australia	Targeted, trapping
T	Delimiting	Plant pathogen	Papaya	Orchard and domestic gardens	Cook Islands	Targeted
U	Delimiting	Plant pathogen and vector	Citrus trees	Orchard and urban	Papua New Guinea	Targeted
V	Delimiting	Insect	Mango	Feral trees, urban and orchards	Northern Australia	Targeted
W	Delimiting	Insect	Fruit fly hosts	All types	Cook Islands	Targeted, trapping

8.2. Case study A. Sugarcane pests in Papua New Guinea, Indonesia and northern Australia

Step 1. Purpose of the survey

New Guinea is the centre of diversity for *Saccharum officinarum*, the source species for high-sucrose genes in commercial sugarcane cultivars, and *Saccharum* species are widely cultivated and naturally distributed through eastern Indonesia and Papua New Guinea (PNG). There are many exotic pests and diseases, some of which occur in Indonesia and PNG, that have the potential to reduce the productivity and profitability of the Australian sugar industry.

The focus of the surveys was to determine the distribution of known insect pests and diseases of *Saccharum* spp. in the PNG–Indonesian–Australian region. This would allow the development of quarantine strategies to limit the spread of these pests.

Step 2. Targeted pest name/s and diagnostic characteristics

All insects and diseases (fungi, bacteria, viruses, phytoplasmas) encountered were collected. In PNG and Indonesia these are mainly endemic, in Australia they are exotic.

Insect specimens were allotted preliminary identification in the field, based on the experience of the surveyors. Some stemborers from the PNG survey were reared through to adults at Ramu Sugar (PNG). Specimens (pinned or in ethanol) were later sent to a specialist for confirmatory identification.

Disease specimens were photographed and allotted preliminary identification in the field, based on the experience of the surveyors. Where the identification was tentative, leaf and/or stem specimens were dried in a plant press or in bottles containing calcium chloride. Fungi were later identified on morphological characters; viruses, bacteria and phytoplasmas were identified using DNA technology.

Step 3. Target host

Cultivated *Saccharum* spp. (*officinarum*, *edule* and commercial hybrids).

Step 4. Alternative hosts

Wild *Saccharum* spp. (*spontaneum* and *robustum*).

Step 7. The area

Four surveys were undertaken in Papua New Guinea, eastern Indonesia and northern Australia and the Torres Strait/Cape York Peninsula region. In PNG, areas visited were Daru, Morehead, Tabubil, Vanimo, Wewak, Manus, New Ireland, New Britain, Lae, Ramu, Popondetta, Alotau and Port Moresby; these covered the extremities of PNG. In Indonesia, Sumba, Flores, Sumbawa, Lombok and Bali were visited. In northern Australia, 19 major coastal and near-coastal settlements from Normanton to Broome were visited. A number of Torres Strait Islands were visited (Mabuiag, Boigu, Saibai, Dauan, York, Murray, Darnley, Thursday, Horne) as well as a number of Cape York communities. Areas such as West Papua (Indonesia) and the Highlands and Bougainville areas of PNG could not be visited because of security concerns.

Most pests and diseases are more active or more obvious towards the end of the wet season when humidity is high and there has been time for the development of populations.

Steps 10 and 11. Site choice and sample size

In all areas, traditional and household gardens containing plantings of *Saccharum officinarum* and commercial hybrid cultivars were targeted. Traditional gardens are planted in community gardens in and around villages. In addition, wild canes growing on roadsides were also inspected.

Due to limited time, villages (3–5 per day) and roadsides within about 20–50 km of airports that were accessible by road were surveyed. In northern Australia, townships were surveyed.

The entire area of each village was searched for *Saccharum* plants. This was usually about 1 ha.

All *Saccharum* plants in the community gardens and communities were surveyed—usually 5–15 stools of sugarcane.

Step 12. Timing of survey

Most sugarcane pests and diseases are more active or obvious towards the end of the wet season—this and the requirements for road and air transport dictated surveys in May–June.

Step 14. Samples collected

The location of each collecting site was determined by GPS and the host species noted.

Insect specimens were collected either as adults or when immature. Most were placed in >95% ethanol (suitable for later DNA analysis) in labelled tubes, while some were pinned after killing. In PNG, stem borers were kept alive and placed in tubes with a food source for rearing and identification at Ramu Sugar. Specimens were taken to Australia (under AQIS permit) for further identification (often by specialists in Australia or overseas). Some samples were duplicated and retained in Indonesia or PNG as reference samples.

Disease specimens were collected as leaf or stem samples. Samples were pressed between sheets of newspaper in a plant press or were cut into small (2 mm by 2 mm) squares and dried in sealed McCartney bottles containing calcium chloride. Material was imported into Australia under AQIS permit (fumigated where necessary). Pressed leaves were deposited in the herbarium of the Queensland Department of Primary Industries and Fisheries and dried leaf samples were sent to BSES Limited's Indooroopilly laboratory for DNA identification of causal organisms.

Comments

It was essential in all surveys to interact with local personnel, usually staff from the national quarantine service or agricultural extension service. These people provided knowledge on local conditions and acted as go-betweens in securing permission to enter villages and collect material. It also helped in technology transfer between the survey team and the local staff.

In many places it was difficult to obtain sufficient newspaper to dry plant material—ample supplies should be taken on all such trips.

Airline regulations meant that tubes containing ethanol had to be packed in a certain way—make sure that this is checked before setting out.

Transport from place to place was by chartered aircraft—this gave much better flexibility and use of time than relying on commercial flights.

References

Magarey R.C., Suma, S., Irawan, Kuniata, L.S. and Allsopp, P.G. 2002. Sik na binatang bilong suka—Diseases and pests encountered during a survey of *Saccharum* germplasm 'in the wild' in Papua New Guinea. Proceedings of the Australian Society of Sugar Cane Technologists, 24, 219–227.

Magarey, R.C., Kuniata, L.S., Croft, B.J., Chandler, K.J., Irawan, Kristini, A., Spall, V.E., Samson, P.R. and Allsopp, P.G. 2003. International activities to minimise industry losses from exotic pests and diseases. Proceedings of the Australian Society of Sugar Cane Technologists, 25 (CD-ROM).

8.3. Case study B. NAQS and SPC early detection and pest list survey design for plant pathogens

Step 1. Purpose of the survey

A broad pest survey to determine baseline data for host and pathogen lists, including organisms of quarantine concern.

Step 2. Targeted pest names and diagnostic characteristics

A wide range of pest species is targeted during these surveys. In general, the pests are identified by surveying all plants showing disease symptoms. For quarantine surveys, target pest lists are developed either through consultants, consultations with stakeholders or literature searches. A quarantine pest was defined as a pest of potential economic importance to the area endangered and not yet present there, or present but not widely distributed and being officially controlled.

The main pests targeted by both NAQS and SPC are citrus canker (*Xanthomonas axonopodis* pv *citri*), banana bunchy top virus, sugarcane smut (*Ustilago scitaminea*), blood disease bacterium, Panama wilt (*Fusarium oxysporum* f. sp. *cubense*) and Huanglongbing ('*Candidatus* Liberibacter asiaticus').

Step 3. Target hosts

A wide range of hosts is targeted, but surveys generally concentrate on economically or culturally important species. The main crops targeted are sugarcane, bananas and citrus plants.

Step 4. Alternative hosts

Weeds are surveyed in the areas visited to detect potential biological control organisms and alternative hosts.

Step 7. The area

This survey is applied to the entire Pacific islands when performed by the SPC and to northern Australia, the Torres Strait islands, Papua New Guinea and Indonesia when performed by the NAQS.

Steps 10 and 11. Site choice and sample size

A wide range of habitats is surveyed. Particular attention is paid to agricultural areas, including broad-scale agriculture and village and house gardens.

As these surveys are detection surveys, time is the factor limiting the number of sites surveyed. The aim is to cover as much as possible of the growing region in each area.

Sites are sometimes chosen on the basis that there are multiple target hosts present in the one location or when farmers or extension officers have reported something new or unusual.

Step 12. Timing of survey

In seasonally wet and dry climates, the surveys are generally timed to coincide with the end of the wet season as the sites were easier to access then and host plants were still growing rapidly. In areas with less-variable seasons it is best to time surveys for when host species are most abundant and crops are growing. The growth of phytoplasmas seems to be favoured in the drier periods of the year.

Step 14. Samples collected

Samples are collected from any surveyed plants showing pests or pest symptoms. Samples can be treated in one or other of three ways. Samples with definite disease signs, i.e. fruiting bodies, are dried and pressed as herbarium specimens. Samples with symptoms are isolated onto fungal growth media or, in the case of inter- and intracellular pathogens, dried over calcium chloride for later analysis.

Comments

Good quality photographs are taken of all the samples, particularly those suspected of harbouring viruses or phytoplasmas. It is useful to take photographs of specimens sent for identification so that there is an image of exactly what has been identified and is held as a voucher specimen. The photographs are also useful in publications.

8.4. Case study C. Pest status and early detection survey for shoot borers in mahogany and cedar trees

Step 1. Purpose of the survey

Pest status surveys and early detection monitoring for shoot borers of mahogany and cedar in forest plantations, woodlots and amenity plantings.

Step 2. Targeted pest name and diagnostic characteristics

Hypsipyla robusta (Moore) (Lepidoptera: Pyralidae)

Common names: mahogany borer, cedar shoot caterpillar

Hypsipyla robusta is indigenous or established in some countries in the Pacific and exotic to others.

Damage symptoms: Insect tunnelling in terminal and lateral shoots of tree causing shoot death and dieback of leaders and branches, and multi-stemming. Early symptoms include wilting of tips and small amounts of frass in a leaf junction. A web containing plant particles and frass usually covers entrance holes. Early instar larvae are brownish-red in colour, and late instar larvae are a distinctive blue with black spots. Adult moths are very rarely seen. The fruits of some hosts are also attacked, damage symptoms being frass and webbing the fruit together in a clump.

Step 3. Target host

Tree species of the subfamily Swietenioideae, family Meliaceae; for example, species of *Toona* (red cedar), *Swietenia* (American mahogany), *Cedrela* (Mexican cedar), *Chukrasia* (Asian mahogany), *Khaya* (African mahogany).

Step 4. Alternative hosts

Xylocarpus spp. (mangroves)

Step 7. The area

Fiji, Vanuatu, Samoa and Tonga

Steps 10 and 11. Site choice and sample size

Sub-areas were defined as forest plantations, woodlots and amenity plantings of Swietenioideae. These were identified by consultation with forestry organisations in each country to determine their location, age and area of plantings.

Surveys were structured to cover the range of susceptible tree species (e.g. *Toona*, *Swietenia*, *Khaya*) and planting types (e.g. plantation, agroforestry, urban) in each country at several geographic locations as resources permitted.

Young plantings (less than 5 years old) of susceptible tree species were chosen because damage symptoms are easier to detect and insect samples more readily obtained. Amenity trees adjacent to air- and seaports receiving international freight were targeted in the surveys because these are considered high-risk sites for incursions by exotic pests. Surveillance was usually concentrated within 1 km of high-risk sites, although plantations of susceptible host species within several kilometres of ports were also generally inspected. Visual inspections of trees were conducted by both roadside cruise ('drive by') and ground transect. If the damage symptoms described above were detected then the tree was examined more closely and attacked shoots were dissected to determine the causal agent. If moth caterpillars were found and their appearance was consistent with that of *H. robusta* larvae, then samples were collected for rearing to adulthood in the laboratory. The moths were then sent to a taxonomist for identification.

Roadside cruise involved driving at speeds not exceeding 15 kph, preferably in a team of two—a driver and an observer. Detection efficiency declines with increasing distance from the road (rarely reliable beyond 40 m) and increasing vegetation density. Periodically during roadside surveys, the team stopped the vehicle and conducted a ground transect of 100 trees through a plantation away from the road.

The number of trees sampled at each site varied with type of planting and survey method. Plantations with good road access were sampled in drive-by surveys allowing the scanning of large numbers of trees for signs of damage. Ground surveys were conducted in all planting types, usually transects totalling 100 trees, the number of transects varying with the size of the planting and according to the time/resources available.

Step 12. Timing of survey

The insect may be present throughout the year but is most active in the hotter, wetter months so sampling was performed at those times.

Step 13. Data collected

Locality, situation (e.g. plantation, amenity), host species, symptoms, incidence (number of trees affected), severity (number of shoots attacked per tree), date, observer and GPS reading.

Step 14. Samples collected

Specimens: 15 cm of shoot containing late-instar moth caterpillar for rearing in the laboratory, additional larvae for preservation, any live pupae for rearing, plant foliage and flowers if required for identification, photographs.

Comments

Permission was sought before entering the survey properties.

8.5. Case study D. Urban pest status survey in Cairns

Step 1. Purpose of the survey

The purpose of the survey was to conduct a pest status survey for a target list of plant pests and diseases in a high-risk urban environment. Cairns city is considered 'high–risk' due to the high level of tourist and trade traffic at this port, and because of the diverse range of horticultural and alternative host flora in this area. The survey also included elements of a monitoring survey, as staff collected information to support PFA status for specific quarantine pests during the course of the survey.

Step 2. Targeted pest names and diagnostic characteristics

Over 100 plant pests listed on the Queensland Department of Primary Industries and Fisheries biosecurity plant pest target list were targeted. The specific number of pests surveyed depended on the horticultural and alternative host plant species encountered during the survey.

Exotic ants, termites and other invertebrate pests were also targeted.

Step 3. Target hosts

The biosecurity plant pest target list identifies approximately 20 different host groups. The main host plants targeted include: mangoes, citrus and other Rutaceae, banana and other *Musa* spp., cucurbits, Malvaceae, grapes and other Vitaceae, and solanaceous crops.

Step 4. Alternative hosts

A range of other horticultural and ornamental host plants was surveyed when encountered by the surveillance staff.

Step 7. The area

The area was defined as the city of Cairns and surrounding suburbs, in Queensland, Australia (Figure D1). The habitats encountered in this area are diverse and include residential backyards, rubbish dumps, industrial and port areas, creek banks, parks and feral stands of horticultural host plants.

Steps 10 and 11. Site choice and sample size

The number of field sites corresponded to the number of contiguous suburbs in the greater Cairns area.

The resources assigned to the project determined the number of sampling sites within a suburb. A team of two scientists surveyed an average of seven sites per day: the time allocated to the project and the number of suburbs to be covered determined how many sampling sites could be completed. Approximately 2.2 sites in each of 38 suburbs were covered, totalling 84 sampling sites.

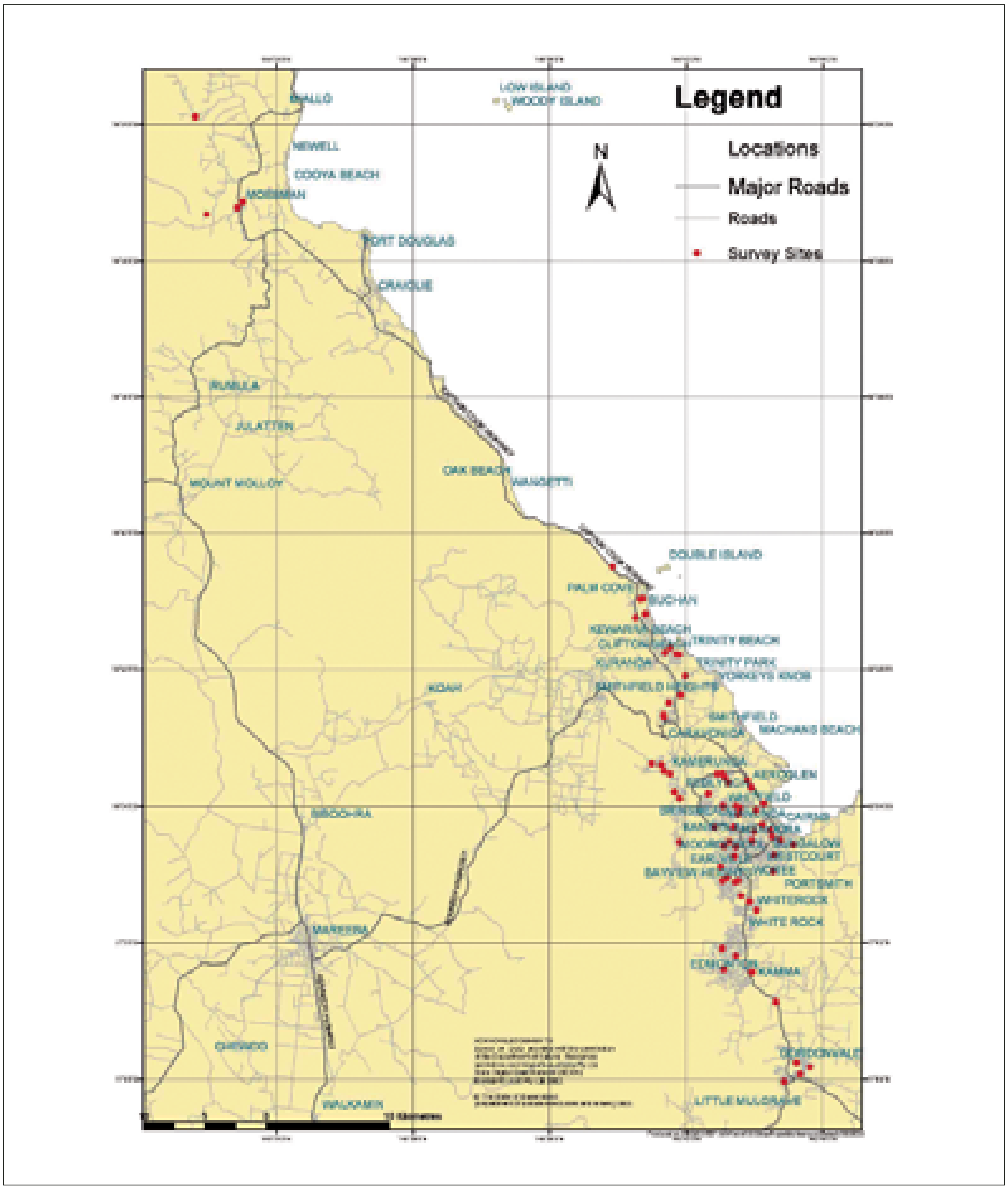


Figure D1. Map of suburbs in and around Cairns, Australia surveyed in September 2003

In order to achieve the most effective surveillance and efficient use of resources, sites were not chosen at random but instead properties with a high number of host plants and a varied host range were targeted. This approach was chosen to increase the likelihood of detecting a target pest.

At each site, all horticultural and alternative host plants were examined. The relatively small size of each site and the fact that host plants are not usually densely planted in residential gardens, often enabled staff to closely examine every host plant. Where there was a high number of plants, such as a large stand of bananas, the group as a whole was surveyed and then a few plants were examined in detail. Over 3760 host plants were surveyed in total, with an average of 11 taxa per site.

Step 12. Timing of survey

This survey is conducted annually. Year-round access can easily be achieved in urban areas which allows scientists the luxury of varying the time of survey each year to enable staff to detect target pests that may have a seasonal life cycle.

Step 13. Data collected

Negative data were collected for pest species as well as recording the presence of regulated and exotic pests. A range of basic host and site location information was also gathered. A sequential site number was used to identify each site. General information was recorded for each site against this number on a survey sheet. The data recorded for each site included the names of observers, date, site description, geographical coordinates, number and type of hosts present, number of hosts examined and number of samples taken. Absence data for specific pests were also recorded on this form.

Step 14. Samples collected

Any suspect exotic species or pests unfamiliar to scientists and causing significant damage were collected for taxonomic identification using the appropriate method. Photographs were taken of collected pests and diseases *in situ* for future reference.

Comments

Urban surveillance has a high element of community involvement, as permission is sought to enter each property. Many of the pest incursions detected in Queensland have resulted from a public enquiry about an unusual insect or sick plant. Urban surveillance and close contact with gardeners is a good opportunity to educate members of the general public about exotic pest species and quarantine awareness. The surveyors took time to talk to property owners, as they can be valuable reporters of exotic pests.

The survey was designed as a pest status survey but data collected could be used to obtain pest free area status to support interstate and international trade.

8.6. Case study E. Pest free area status survey for khapra beetle in stored grain

Step 1. Purpose of the survey

To maintain Australia's pest free status for the khapra beetle

Step 2. Targeted pest names and diagnostic characteristics

Targeted were the khapra beetle (*Trogoderma granarium*) and the warehouse beetle (*Trogoderma variabile*). The khapra beetle is the world's worst pest of stored grain. It is not found in Australia and many grain export markets would be lost overnight if this pest were found here. The warehouse beetle is present in the midlands region of Western Australia. The main significance of the warehouse beetle is that it can mask the presence of the khapra beetle.

Infestations are usually noticed through cast larval skins. Identification requires the dissection of mouthparts, and suspect beetles are sent to a taxonomist for identification.

Step 3. Target host

Grain, cereals and products including wheat, barley, oats, rye, maize, rice, flour, malt, and noodles.

Step 4. Alternative hosts

None were surveyed.

Step 7. The area

Grain export terminals, storage sites and grain processors in Western Australia, where the warehouse beetle is known or at risk of infesting.

Steps 10 and 11. Site choice and sample size

Field sites were selected in areas at risk of infestation. The number was determined by the storage sites involved, which was approximately 130 across 30 towns. The storage sites included commercial buildings containing grain and grain products, and commercial grain storage facilities.

Sticky traps (see next page) were set up within each grain store. Also, up to five baiting sites were chosen near food sources in large buildings, and one site in small buildings (such as shops).

Some sentinel pheromone traps were placed on farms on an ad hoc basis, targeting farms with poor hygiene.

Step 12. Timing of survey

Trapping was performed during the warmer summer months, when beetle activity is at it highest (December to March). Traps are effective for 2 months and so were replaced at the end of January. In warmer climates, the beetles could possibly be active all year and so would require continuous surveillance. At seaports, the trapping was continuous.

All traps were checked every 2 weeks.

Step 13. Data collected

Recorded were trap identifier, date, location, property name, property type, nearby food sources and comments including trap position within the storage site.

Step 14. Samples collected

Sticky traps with pheromone lures were used. These can attract beetles from a distance of 5 km. The lures attract native *Trogoderma* beetles, warehouse beetles and khapra beetles. Because khapra beetles do not fly, traps are placed at ground level.

As the pest was not found, no results were recorded. Null records are not kept but it is intended that they will be in future surveillance.

References:

Emery, R., Dadour, I., Lachberg, S., Szito, A. and Morrell, J. 1997. A final report prepared for the Grains Research and Development Corporation. The biology and identification of native and pest *Trogoderma* species. Project number DAW 370. South Perth, Agriculture Western Australia.

Banks, H. J. 1990. Identification keys for *Trogoderma granarium, T. glabrum, T. inclusum* and *T. variabile* (Coleoptera: Dermestidae). Black Mountain, Canberra, Australia, CSIRO Division of Entomology.

Comments

Surveys should be more rigorous (structured rather than sentinel), ongoing, nationally coordinated and the results entered in a database.

The traps could be inspected more frequently to allow rapid action if an incursion occurs. Beetles can be carefully removed from traps without destroying the trap and sent off for identification.

8.7. Case study F. Pest free area status survey of Queensland fruit fly and Mediterranean fruit fly

Step 1. Purpose of the survey

To seek PFA status in order to gain international market access.

Step 2. Names of targeted pests and their diagnostic characteristics

Mediterranean fruit fly (Medfly)—*Ceratitis capitata* (Wiedemann); Queensland fruit fly (Qfly)—*Bactrocera tryoni* (Froggatt).

Medfly is an exotic species that has a restricted distribution in Western Australia, with permanent populations existing only in the southwestern part of that State (Figure F1). This area is over 2000 km from the Riverland, Riverina and Sunraysia PFAs.

Qfly is a native species and originally had a limited distribution around southeastern Queensland. Qfly now exists in permanent populations along coastal strips of the eastern seaboard, extending up to 300 km inland in Queensland, through New South Wales and tapering off to limited areas in northeastern parts of Victoria.

Any detections of economically damaging fruit fly species are viewed as serious.

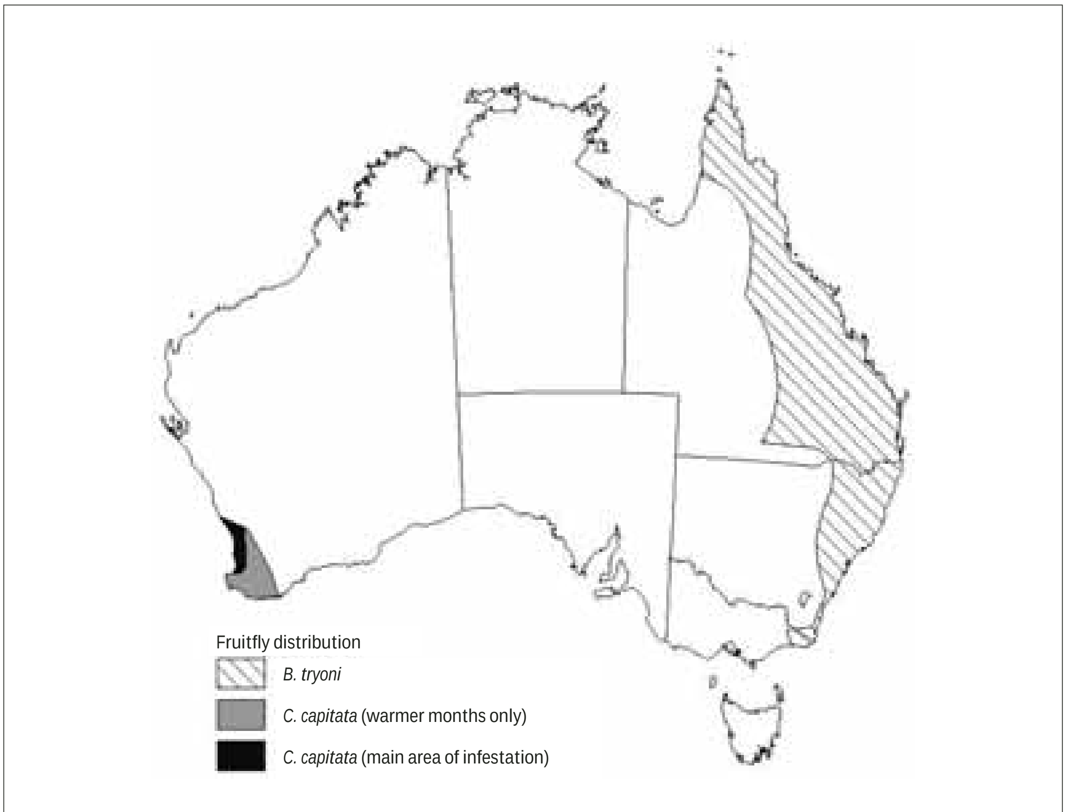


Figure F1. Distribution map of QFly *(Bactrocera tryoni)* and Medfly (*Ceratitis capitata)*

Step 3. Target host

Fruit trees: apple, pear, apricot, nectarine, peach and citrus.

Step 4. Alternative hosts

None were surveyed.

Step 7. The area

The area comprises the Riverland, Sunraysia and Riverina regions in Victoria and New South Wales, Australia (Figure F2). All three regions and the land surrounding them are geographically isolated by significant distances from areas of Australia infested with permanent populations of Medfly and Qfly.

Medfly and Qfly do not occur in the area and they are not capable of naturally dispersing to these pest free areas (PFAs) from infested areas, due to the hostile climatic conditions experienced in the PFAs and surrounding lands. These conditions are sufficiently detrimental that the risk of fruit fly establishment in these areas is marginal.

Any introductions of Medfly and Qfly from infested areas into the PFAs could only be through transport by humans. The illegal transportation of infested fruit from fruit-fly infested areas by travellers in private vehicles is believed to be the main potential source of introduction of fruit fly into the PFAs.

Transport of host fruit by humans into the PFAs is strictly controlled by State legislation. Additional phytosanitary measures are also used to prevent the introduction and spread of these fruit flies. There is therefore minimal risk of fruit flies entering and establishing in these PFAs.

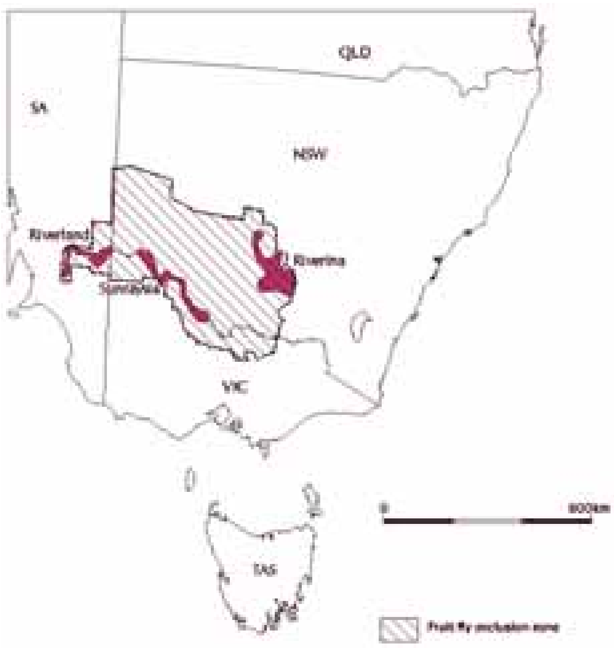


Figure F2. Regions (in red) seeking pest free area status

Steps 10 and 11. Site choice and sample size

Permanent trapping grids are used. Traps are placed at a higher density in urban areas than in non-urban horticultural areas because urban areas are considered to carry a higher risk for the entry and establishment of fruit fly. Sentinel traps are used in major towns.

In the PFAs, the trap sites are placed in a grid with:

- 1 trap site every 400 m in urban areas
- 1 trap site every 1 km in non-urban horticultural areas where host production occurs.

Step 12. Timing of survey

Traps are monitored throughout the year; weekly during late spring, summer and early autumn when trees are producing fruit, and fortnightly at other times.

Step 14. Samples collected

Separate traps were maintained for Medfly and Qfly but the traps are placed at the same site. Each trapping site consists of:

- one Lynfield trap charged with *Cue-lure* plus maldison for Qfly
- one Lynfield trap charged with *Capi-lure* plus dichlorvos for Medfly

Traps were placed in the best vegetative cover available. During the southern hemisphere spring/summer, traps were placed in host trees of apple, pear, apricot, nectarine or peach wherever possible, while in autumn and winter, citrus trees were used.

Traps are located within the canopy of host trees bearing fruit, approximately half the distance from the trunk to the outer edge of the foliage, and at least 1.5 m above the ground. If no fruiting trees are available, the traps are placed in trees with similar foliage (i.e. broadleaved trees). Traps are located at least 3 m apart at each site.

Cue-lure traps are recharged twice per year in spring (September) and summer (January). The entire trap (base and lid) is replaced every 12 months in spring (September), unless a trap is damaged, in which case it is replaced immediately.

Capi-lure traps are recharged four times per year in spring, summer, autumn and winter. The entire trap (base and lid) is replaced every 12 months in spring (October).

All insects from traps are examined and suspect fruit flies forwarded to a reference entomologist for positive identification. Any specimens are placed in individual plastic vials and a label attached listing details of the trap number, date and other details. If there is doubt about the identity of suspect flies, the specimen is sent to a taxonomist for positive identification.

Comments

AQIS is responsible for ensuring that the permanent fruit-fly monitoring program in the Riverland, Riverina and Sunraysia PFAs is conducted in accordance with trading partner requirements. The State government departments are responsible for the routine maintenance and management of the fruit fly monitoring programs in their States (e.g. establishment and servicing of traps, identification of flies, implementation of eradication campaigns). AQIS conducts audits of the fruit-fly monitoring programs to ensure compliance, and the State authorities are responsible for ongoing internal audits of their activities and procedures.

The State departments have the responsibility and legislative power for declaring outbreaks. They are required to inform AQIS of the details of any outbreak and the required suspension area.

AQIS is responsible for the subsequent notification of any outbreaks to trading partners. As for other national plant protection organisations, AQIS has the responsibility and legislative power for export certification of host produce originating from the PFAs. When outbreaks do occur, AQIS will not permit exports from those designated outbreak areas.

8.8. Case study G. Pest free area status for dodder weed

Step 1. Purpose of the survey

The purpose of this survey was to demonstrate if the Ord River Irrigation Area (ORIA) in the north of Western Australia was free of weed pests belonging to the genus *Cuscuta* (dodder weed). This information was needed to support negotiations for market access to the USA market for Niger seed, which is used in caged bird seed mixes. US regulations insist that Niger seed be steam cooked to kill any weed seeds present, especially those of *Cuscuta* species. The US Quarantine Service APHIS agreed that ORIA Niger seed could be imported into the USA without steam cooking if the area was demonstrated to be free of *Cuscuta*.

All recorded locations of the pests were researched. It was unknown in the survey area. The nearest known *Cuscuta* species were 1000 km to the south and 200 km east-southeast. To the south of 18°S latitude there were scattered populations of *Cuscuta* species. Surveys in 1993 and 1994 extended the range of *Cuscuta* species in Western Australia to within 300 km of the ORIA.

Step 2. Targeted pest name and diagnostic characteristics

Cuscuta species. This parasitic plant has neither leaves nor chlorophyll. Its fine stems form a tangled mass, clinging to herbaceous plants such as legumes, tomatoes and capsicums by means of small suckers. The dodder uses these suckers to draw nutrients from the hosts, which become stunted and discoloured.

Step 3. Target hosts

Niger seed (*Guizotia abyssinica*), hybrid sorghum (*Sorghum* sp.) and pearl millet (*Pennisetum glaucum* hybrids). These annual crops are grown in the dry season. They are generally planted in April–May and harvested in August–September.

Step 4. Alternative sites

Other places that the weed could possibly grow and that were relevant were:

- amongst any other crop (bananas, mangoes, maize, chickpeas, melons and leucaena)
- in non-cropped wet habitats (springs, drainage ditches, water-supply channels, gardens, lake margins, drainage swamps, paddock margins and river margins)
- road verges.

Step 7. The area

The ORIA is serviced by the town of Kununurra. The area was defined for this survey as the Kununurra townsite and that area of the Ord River Irrigation Project that is used for irrigated agriculture. It covers approximately 5400 square kilometres.

The ORIA is in the semi-arid tropics. The climate is hot and wet in summer (wet season) and warm and dry in the winter (dry season). Average annual rainfall is 787 mm, falling mostly between December and March with the maximum temperature sometimes exceeding 40°C. Maximum temperatures in the dry season average 32°C and minimums 15°C.

This area consists largely of black cracking-clay plains that are used for intensive irrigated row-crop agriculture. There are some areas of red loams and sands and these are either row-cropped, or under tree crops.

Steps 10 and 11. Site choice and sample size

The number of sites was influenced by the large area and the fact that the species are hard to see. The number of sites was designed to cover the entire area. The level of surveying intensity varied with the site type.

1. Surveying of all Niger, sorghum and pennisetum (millet) crops intended for export to the USA. These were examined at an intensity of one site per 10 ha. N = 20.
2. One site was surveyed for each type of other crops (bananas, mangoes, maize, chickpeas, melons and leucaena) and all the non-cropped wet habitats (springs, drainage ditches, water-supply channels, gardens, lake margins, drainage swamps, and paddock and river margins). N = 30.
3. When travelling in the area, all road verges were observed by a drive-by survey.

At each sampling point, surveying consisted of walking a 500 m transect in crops (or twice as far around the edges of dense vegetation such as sorghum and bananas) inspecting to 1 m to the left and right of the transect.

If the area surveyed was a low crop or consisted of uncleared bush, a 500 m transect in zigzag pattern was used to survey as much of the crop or site as possible.

Sites in the crop were targeted if there were areas that were uneven or showing yellowing, especially near irrigation supply and drainage sites of crops.

Step 12. Timing of survey

The area was surveyed for *Cuscuta* species once only in the wet season (March–April) and once during each subsequent dry season when Niger and the hybrid pennisetums and sorghums are growing under irrigation.

The frequency of surveying was specified by the trading partners. Export of Niger to the US has ceased for the time being, but as part of the NAQS survey for a wide range of pests in northern Australia, *Cuscuta* is searched for every 6 months in a less structured manner.

Step 14. Samples collected

No samples were collected as the pest was not detected.

Step 21. Reporting

The survey results were reported to AQIS after every survey.

Comments

Legislative changes proposed to support the pest free area status:

- prohibit the importation of *Cuscuta* species into the ORIA under the *Plant Diseases Act 1914*
- declare *Cuscuta* species a noxious weed in the ORIA under the *Agricultural and Related Resources Protection Act 1976*, which will allow eradication of possible future incursions.

8.9. Case study H. Pest free area status for mango pulp weevil and mango seed weevil

Step 1. Purpose of the survey

To seek market access into Australia for mangoes.

Step 2. Targeted pest names and diagnostic characteristics

Mango pulp and seed weevils, which are similar in appearance but attack different parts of the fruit. Because there are no external signs of the weevil attack, fruit have to be cut open and examined. The pulp weevil larvae form distinctive brown chambers within the flesh.

Step 3. Target host

Mango

Step 4. Alternative hosts

None examined.

Step 7. The area

Guimaras Province, Republic of the Philippines, which consists of a group of islands. Some 8% of the total land is dedicated to mango production. The islands are geographically isolated from other islands by sea straits.

Steps 10 and 11. Site choice and sample size

A census of the province identified over 97,000 mango-bearing trees and the location of each. This permitted randomised selection of the target trees. The sampling sites were stratified by municipality, and then divided by the distribution of cultivars and the management practices.

The sample size (approved by an Australian government statistician) was such that if either the mango pulp weevil or the mango seed weevil were present with a tree infestation rate of 1% or greater and if 15% of the fruit on an infested tree were infested, there would be a greater than 95% chance of detection during the survey. The sample size required 5% of all known mango-bearing trees in the province to be surveyed and that 10 fruits be collected from each.

Trees were visited for suitability before the survey and painted with a number.

Step 12. Timing of survey

The survey was conducted from February to May 1999. Production is throughout the year, but peaks during December–May and in March and April.

Step 13. Data collected

The grower or agent was interviewed on aspects of cultivar, horticultural practices, tree management, yield and pest incidence.

Step 14. Samples collected

Ten mangoes, older than 65 days after flower induction, were collected per tree. At 5%, the sample size needed was 4857 trees, collecting 48,570 mangoes for investigation. At least two mangoes were to be collected from each of the four quadrants around each tree.

Mangoes were bagged and labelled then sent to a laboratory for examination. Mangoes were examined externally then dissected to search the pulp and the seed for all pests, but with emphasis on the target pests.

Fruit collecting was considered to be more efficient and effective than sticky traps, visual observations, beating of branches and debris examination.

8.10. Case study I. Insect pests of food plants in Aboriginal communities in the Northern Territory

Step 1. Purpose of the survey

The purpose was to survey the Aboriginal communities of Yirrkala and Garrthalala, the town of Nhulunbuy and nearby native vegetation for exotic insect pests.

Step 2. Targeted pest names and diagnostic characteristics

A targeted pest list was used, based on insect pests that are not yet present in Australia but are found in neighbouring countries. The list included 56 high-priority and 24 moderate-priority species. These pests attack primarily food crops but could possibly survive on related plants.

Step 3. Target host

A group of priority plants that are important sources of food and other resources in northern Australia was surveyed. These were sugarcane, banana, citrus, mango, cotton, grape, grain sorghum, cucurbits, maize, pasture legumes and grasses, *Eucalyptus* spp., *Acacia* spp. and palms.

Step 4. Alternative hosts

Native plants belonging to the same genus or family as the priority plants were also targeted, especially if the targeted insect was known to be polyphagous.

Where time permitted, other food plants, especially native food-plant species important to local people, were also surveyed.

Step 7. The area

The survey was confined to the mining town of Nhulunbuy (population 2000), and the Aboriginal communities of Yirrkala (population approximately 1000) and Garrthalala (population approximately 30). Nhulunbuy is a coastal town on the north-eastern tip of Arnhem Land in the Northern Territory of Australia. An uninhabited coastal site near Garrthalala, Murjbi, was also surveyed following reports of a foreign vessel in the area previously.

Nhulunbuy supports a wide variety of plant species in backyard settings, usually not in close contact with native vegetation. Yirrkala also has some backyard plantings, though not as substantial as those in Nhulunbuy, but native plant species growing close to commercial plant species are more common. There is a 5 ha banana plantation at Yirrkala. Garrthalala has few commercial plant species and is totally surrounded by native vegetation. Murjbi is an uninhabited and relatively undisturbed site.

Access to backyards in Nhulunbuy and Yirrkala was dependent on the homeowner being available to grant permission. Access to yards in Garrthalala was easily negotiated with the community elders. Garrthalala was a two-hour drive from Nhulunbuy and Murjbi, and a further hour on a narrow, dirt track. Access to Aboriginal lands required permission from the local land council and was greatly facilitated by the participation of local people in the survey.

Steps 10 and 11. Site choice and sample size

Backyards growing target hosts were found by asking local people and by driving and walking around town looking into yards. Native vegetation bordering the communities was targeted.

Sites were chosen largely as they were located. Due to time restrictions, the proportion of yards surveyed was inversely proportional to the size of the community. In Garrthalala, 100% of commercial species were surveyed, while in Nhulunbuy and, to a lesser extent, Yirrkala, lower proportions were included.

The number of plants examined varied according to the site. All plants in the community gardens surveyed were examined. In the banana plantation at Yirrkala, banana plants growing around the edge of each block, and those along a transect through the middle of the plantation, were surveyed. Native vegetation growing on the edge of the communities was surveyed.

Step 12. Timing of survey

The survey was conducted in December to coincide with the start of the wet season—before roads are cut by rainfall but after plants had started growing. It also allowed for inspection of mango fruits.

Step 13. Data collected

At each community, a list of food plants present was compiled.

Step 14. Samples collected

All insects collected were identified in the field as far as possible, usually to family level. Only those specimens which could not be definitively identified as not being a targeted species, or those of uncertain identity yet causing significant damage to plants, were retained. Interesting or unusual species or symptoms were photographed.

8.11. Case study J. Early detection survey for sugarcane smut

Step 1. Purpose of the survey

Sugarcane smut is a serious disease of sugarcane that can cause yield losses over 30% in susceptible cultivars. The disease was found for the first time in Australia in July 1998 in the Ord River Irrigation Area of Western Australia. An initial rapid survey in eastern Australian sugarcane crops and a review of disease inspections conducted in 1998 failed to locate smut in eastern Australia.

More extensive sugarcane smut surveys throughout Queensland and New South Wales were undertaken in 1998–99 and 1999–2000 to determine the presence or absence of sugarcane smut in eastern Australia and enable appropriate quarantine or incursion management decisions to be made to reduce production losses.

Step 2. Targeted pest name/s and diagnostic characteristics

Pathogen: *Ustilago scitaminea* H & P Sydow

Disease: Sugarcane smut

Diagnostic symptoms: Highly characteristic black whip-like structures (sori) are produced from the heart of the shoot. Whips can be from a few centimetres to over one metre in length (Figure J1). The disease also causes severe stunting, profuse tillering and thin grassy stalks. Experienced plant pathologists can provide reliable diagnoses of the disease.

Figure J1. Characteristic appearance of sugarcane smut whips on sugarcane

Had a suspect infected plant been found, the spores of the fungus would have been sent to the Queensland Department of Primary Industries and Fisheries Herbarium for confirmation of identification, using available DNA methods to identify the fungus.

The disease primarily infects buds and may remain dormant until the buds germinate. Because of this, it may be 6–12 months before any symptoms develop, with the disease going undetected until it reaches sufficient intensity for a survey to have a reasonable chance of detecting an infected plant.

Step 3. Target host

Commercial sugarcane (*Saccharum* spp. hybrids)

Step 4. Alternative hosts

None surveyed.

Step 7. The area

Commercial sugarcane fields in eastern Australia. Generally, these areas have good access and are relatively flat.

Steps 10 and 11. Site choice and sample size

Mill maps were used to provide details on susceptible varieties, farm numbers, block numbers, variety, and crop class. Each field of sugarcane was considered a potential sample site.

Fields were selected at random, although high-risk fields (e.g. on farms where residents had visited the Ord River area) and fields of varieties known to be highly susceptible were also targeted. Ratoon cane was preferred for smut inspections because of longer exposure to possible infection.

There were several factors that determined which parts of the crops could be accessed. The height of cane crops, the narrow laneways with overhanging crops, wet and boggy conditions during summer and the distances to be covered required specialised transport. Both two- and four-wheeled motorbikes were used. The four-wheeled motorbike had trouble fitting through interrows less than 1.5 m apart or where the cane was older than third ratoon. However, it was the best infield survey transport in most mill areas. Each bike had a cage designed to prevent face and eye injury from sugarcane leaves. In some areas, over-row spray machines were used to inspect cane from above the crop; safety harnesses and rails were put in place. Cane was also inspected from the tray of a utility along headlands and winch tracks, and by walking rows in some areas.

A target of 1% of the eastern Australian crop was set as a minimum coverage area for the first year. The potential infection area per mill was comprised of the total area harvested for milling that season, the area harvested for plants and the area left as stand-over. These three estimates were combined to produce the area under cane and referred to as the potential survey area. The required inspection rate per mill area was 1% of the area under cane. The 1% was covered by inspecting 10% of blocks in a mill area, then 10% of the rows within those blocks.

The 1998–99 season was the wettest season for many years in most districts of the Queensland and New South Wales sugar industry. This wet weather hampered the survey in many districts and so only 0.76% of the area was surveyed. A drier season in 1999–2000 and an earlier start (September) allowed a greater area to be surveyed that year.

The combined sugarcane smut surveys covered an area of 15,000 ha or 3.75% of the eastern Australian crop over 2 years. This gave approximately a greater than 95% chance of detecting a 0.1% infection (assumptions: 100,000 × 4 ha units = total 400,000 ha; 3750 × 4 ha units were inspected).

Step 12. Timing of survey

November–March in the first year; September–March in the second year.

The timing follows harvest of the preceding plant (first year after planting) or ratoon (subsequent regrowth) crop. This means that the fields are accessible and there has been some time for the plants to grow and allow the smut infection to develop the 'whip' that is the indicator to the survey team.

Step 13. Data collected

Inspection results were recorded in a Microsoft Excel database with the following information: mill area; farm name; farm number; inspection date; block number; area of block; cultivar; crop class; actual area inspected; diseases noted.

Step 14. Samples collected

No samples were collected as the pest was not found. If the pest had been found, the plants were not to be touched but marked for further inspection. All inspectors had been briefed on symptoms of sugarcane smut and carried photographs of the disease.

Reference

Croft, B.J., Magarey, R.C. and Smith, D.J. 1999. Survey of sugarcane in eastern Australia for sugarcane smut. BSES Project Report PR99003.

Comments

As a contingency for a smut incursion, a trailer with a set of protective clothing and disinfection equipment was carried to the sites being inspected. The trailer contained:

- a Spitwater cold water petrol HP152—a high-pressure water cleaner (2000 psi; 11 litres/min)
- 200 L plastic drum and holder
- toolbox
- 20 L and 10 L jerry cans for motorbike and pressure cleaner fuel (unleaded)
- 5 L container concentrated truck wash
- box of 20 heavy duty disposable overalls.

The washing equipment was used to wash all dirt, mud and seeds from the motorbikes, utility and trailer between mill areas. If the bikes became very muddy or the blocks within a farm had heavy weed infestations, the bikes were washed thoroughly at the farmer's shed before moving to another farm.

Personal disinfection equipment was placed in a backpack as a precautionary measure in case of smut incursion. This equipment was referred to as a smut incursion kit (SIN kit) and included:

- stiff-bristled scrubbing brush to remove mud and dust
- spray bottle for application of 70% alcohol
- screwdriver for removal of mud in soles of shoes
- 1 L of 70% alcohol (diluted methylated spirits)
- spare set of clothes (pants and shirt)
- packet of heavy duty garbage bags.

8.12. Case study K. *Pseudomonas* in rice

Step 1. Purpose of the survey

Early detection survey

Step 2. Targeted pest name and diagnostic characteristics

Targeted was the bacterium *Pseudomonas*. Early symptoms can be easily confused with those of sheath blight. The lower sheath-leaf of infected seedlings turns yellow, becoming brown and then dark brown. When severe, the whole sheath becomes necrotic. The grains discolour, become deformed, or are empty. Symptoms are generally observed about 80 days after sowing.

Step 3. Target host

Rice

Step 4. Alternative hosts

None examined.

Step 7. The area

Parit Buntar, northern Perak State, Malaysia. This is one of the main granary areas in Peninsular Malaysia. The area under rice cultivation in Parit Buntar is about 20,000 hectares.

Steps 10 and 11. Site choice and sample size

One sampling block covers approximately 40–100 hectares depending on the terrain and infrastructure (e.g. irrigation canals, roads etc.) of the field. Each sampling block was divided into 10 sub-blocks. At each sub-block, 10 sampling points (15–20 tillers/each point) are randomly chosen to monitor the status of pests and diseases. Generally, the coverage in terms of area is about 5–10%, depending on the availability of resources such as scouting staff and vehicles.

The survey was performed in farmers' fields. In each field, a diagonal line was laid down and, walking along that line, one clump of plants every 10 steps was checked. One clump generally measured 15 × 15 cm and consisted of about 20 tillers.

Step 12. Timing of survey

There are two annual planting seasons, September–February and March–July. Sampling was carried out 70 days after planting in both seasons, when symptoms were present. Surveys usually took about a week to complete.

Step 14. Samples collected

The lower sheaths are examined for symptoms in all 20 tillers at all the 10 points to determine the severity status. Leaf sheaths with suspect symptoms were collected for laboratory culture and identification.

Reference

Saad, A., Jatil Aliah, T., Azmi, A.R. and Normah, I. 2003. Sheath brown rot: a potentially devastating bacterial disease of rice in Malaysia. International Rice Conference, Alor Setar, Kedah, Malaysia, 2003.

8.13. Case study L. Monitoring survey of giant wood moth on eucalypt and teak trees

Step 1. Purpose of the survey

Monitoring for stem symptoms of wood moth attack in large monoculture plantations of eucalyptus to determine changes in population size or distribution and to determine damage levels. This will assist forest managers in their decision-making on the need to implement pest management measures.

Step 2. Targeted pest name and diagnostic characteristics

- *Endoxyla cinerea* (Tepper) (Lepidoptera: Cossidae) (formerly in the genus *Xyleutes*).
- Common name: giant wood moth.
- Indigenous to Australia.

Damage symptoms: The insect affects trees 2 years of age and older. Tunnelling by the moth larva within the stem/trunk of a tree produces a swelling of the stem around a small entrance hole. Coarse frass or sawdust is often evident around the base of the tree. Before the adult moths emerge in midsummer, a large, circular exit hole (3–5 cm diameter) is formed above the smaller entrance/feeding hole. When the moth emerges, its empty pupal skin is usually left protruding from the hole, another indicator of wood moth attack.

Visual inspections of trees are conducted by ground transect. If stem swelling and/or coarse frass is observed, then the tree is examined more closely to find an entrance/feeding hole (distinguishing the damage from that of other agents that can cause swelling, such as fungal canker or physical wounding). If it is necessary to confirm identification, smaller trees are usually felled and a section of stem containing the larva is collected for rearing in the laboratory to the adult moth. The moth is then sent to a taxonomist for identification. Larvae of the giant wood moth may reach 15 cm in length and 3 cm in diameter.

Step 3. Target hosts

Eucalyptus grandis (rose gum) and hybrids, *Eucalyptus dunnii* (Dunn's white gum), *Eucalyptus tereticornis* (forest red gum), *Eucalyptus camaldulensis* (river red gum).

Step 4. Alternative hosts

Several other species of native eucalypt not used in commercial plantations in Queensland and New South Wales.

Step 7. The area

Coastal areas of Queensland and northern New South Wales, Australia.

Steps 10 and 11. Site choice and sample size

The districts were industrial forest plantations of the host species listed. These were identified by consultation with known commercial tree growers in Queensland and New South Wales to determine location, age and area of plantings.

Surveys were structured to cover a range of susceptible tree species and age classes across the geographic range of the pest. Larger plantation areas were targeted over small ones because this was more time and cost effective.

Because the insect affects trees 2 years of age and older, younger plantings were excluded. Plantations of host tree species aged 2–3 years were sampled before the first thinning to determine the initial incidence and severity. The impact of wood moth attack is generally most severe for these age classes (tunnelled trees are susceptible to breakage by wind and cockatoo predators seeking the larvae). Sampling was carried out across the range of older age classes but was generally less intensive than for younger plantings.

Ground surveys, although much more effective than roadside cruise in detecting giant wood moth symptoms, are slower to complete and this limited the area of forest that could be sampled. There were four different approaches used: surveying lots of 100 tree transects; surveying fixed-length transects (e.g. 100 m long × 10 m wide strips) per unit area of forest; surveying every 10th row in a plantation compartment; or surveying five lots of 20 tree plots (4 rows × 5 trees) per compartment. An advantage of fixed plots was that they could be sampled over time to show population changes. Paired observers were best for these surveys as they can cover two sides of a tree.

Step 12. Timing of survey

Surveys are commonly carried out during winter when the exit holes of moths are still visible and when new attack can be easily assessed. Where specimens are required for identification, sampling is best conducted in mid-summer when circular exit holes are visible but late-instar larvae or pupae are still present in the stem. Surveys in late summer can also be effective because pupal skins protruding from wood moth holes aid detection.

Step 13. Data collected

Locality, plantation compartment, host species, planting date, symptoms, incidence (number of trees affected), severity (number of attacks per tree), date, observer, GPS reading.

Step 14. Samples collected

Stem lengths of 30–50 cm containing late-instar larvae or preferably pupae for rearing in the laboratory were collected, along with additional larvae for preservation, plant foliage and flowers if required for identification, and photographs were taken.

Comments

This technique is applicable for surveys of other stem-boring wood moths such as *Xyleutes ceramica*, the beehole borer of teak (*Tectona grandis*) in Asia, and could be combined with surveys for other borers such as *Phoracantha* spp. longicorn beetles.

8.14. Case study M. Monitoring survey for damping-off in garden nurseries

1. Purpose of the survey

The purpose was to monitor for damping-off in nurseries. Damping-off is the most serious disease affecting forest plantation nurseries in the tropics. Losses of up to 100% of a season's sowings have been reported. While the disease may destroy the entire nursery stock during one rainy season, it may not be important in other years.

This case study is written to provide guidance as to how to survey for damping-off in nurseries.

2. Targeted pest name and diagnostic characteristics

Damping-off fungi may be endemic in a nursery without causing damage until environmental conditions change to favour the pathogen and disease development but not early seedling growth. Such conditions are, for example, densely sown seedbeds or trays of seedlings, high soil moisture and humidity, over-watering, excessive shade, and poor ventilation. Damping-off can occur within 2 weeks of seed germination causing large-scale mortality. Viable seeds are killed before the shoot emerges from the soil. Some seedlings that emerge appear to collapse and die. Affected seedlings develop water-soaked, constricted stem tissue at soil level causing them to fall over and die. Dead and dying plants occur in irregular spreading patches. Once the infection occurs, it can spread very quickly and kill a large number of seedlings within a few days.

Damping-off problems very often develop into root-rot problems after the stem and some of the roots have begun to become woody, so that it becomes impossible to separate the two. Root-rot symptoms are manifested as stunting of seedlings, top dieback, chlorosis and premature defoliation. Roots are discoloured and/or decayed.

Description of pests

Many indigenous soil-borne fungi are common damping-off fungi that invade the succulent stem tissues. These include fungi in the genera *Cylindrocladium*, *Fusarium*, *Pythium*, *Phytophthora*, *Rhizoctonia* and *Sclerotium*.

Pre-emergence damping-off occurs as a result of fungus attack of the radicle before seedling emergence from the ground. Post-emergence damping-off occurs when the fungus attacks the base of the seedling stem after it emerges from the ground.

Identification of the fungus or fungi causing damping-off should be carried out by a forest pathology or plant pathology laboratory because a wide and diverse group of fungi may be involved.

Step 3. Target host

Damping-off is not host-specific and commonly occurs throughout the world wherever seedlings grow, in greenhouses, nurseries and natural areas.

Step 4. Alternative hosts

See Step 3.

Step 7. The area

This survey can be applied to any garden nurseries.

Steps 10 and 11. Site choice and sample size

A general monitoring survey should be carried out in any nursery or greenhouse where large numbers of seedlings are raised for a planting program. If there is any knowledge of the occurrence of the disease in a particular area, such locations should also be included in the survey.

Seedlings in seedbeds, nurseries and sometimes in the natural forest may be affected. Densely sown seedlings may be more susceptible, especially during the wet season or when over-watered or raised on substrates with a high organic-matter content.

If the nursery is relatively small and adequate personnel are available for the survey, then a complete survey of the newly sown beds in the nursery could be carried out. However, in large nurseries, or where personnel are limited, about 10% of the newly sown nursery beds in the nursery could be surveyed.

Step 12. Timing of survey

The survey should be carried out about a week after sowing, just as the seedlings emerge from the soil. This is the time when symptoms will be evident if damping-off is present.

Step 13. Data collected

In newly sown seedling beds, a visual assessment should be made of the extent of the disease in each bed, as it is impossible to count the number of individual seedlings affected. Large nursery beds can be divided into sectors, quadrants or strips for visual assessment. Assess disease incidence as follows:

Disease incidence	Symptoms	Score
Nil	Nil	0
Low	Up to 25% of seedlings affected	1
Medium	25–50 % of seedlings affected	2
Severe	More than 50% of seedlings affected	3

The disease scores as determined above and the number of seedling/nursery beds surveyed are used to calculate the disease index, which is an indication of the severity of the disease. The disease index is determined as follows:

Disease index = $[(na \times 0 + nb \times 1 + nc \times 2 + nd \times 3) \div (N \times 3)] \times 100$

where:

na = number of beds with score 0

nb = number of beds with score 1

nc = number of beds with score 2

nd = number of beds with score 3

N = total number of beds assessed or in the nursery.

Data to be collected include the total number of seedbeds in the nursery, quantity of seeds sown per seedbed, date of sowing and emergence, frequency of watering, shading conditions, and any disease observations made by the staff of the nursery.

Step 14. Samples collected

Diseased specimens, i.e. the entire infected seedling, should be collected for isolation and determination of the associated fungi.

8.15. Case Study N. Monitoring for root diseases in hardwood plantations

Step 1. Purpose of the survey

The purpose is to monitor root and butt rot in hardwood plantations, including some conifers such as hoop pine. Root diseases are widespread in nature and require special attention at all levels of planning. Since they affect forest productivity, recreational safety, and biodiversity, monitoring is important so that appropriate planning and management measures can be undertaken.

This case study is written to provide guidance as to how to survey for root diseases in trees, using root rot as an example. The survey is described in Old et al. (1997).

Step 2. Targeted pest name and diagnostic characteristics

In plantation forests, *Phellinus noxius* (Corner) G. Cunn. root-rot disease is characterised by slowly enlarging patches of dead and dying trees. The foliage of affected trees is usually paler green, sparse and much reduced in size. The condition of the tree crown shows a general decline and the overall growth rate is poor. Young shoots may wilt, and some of the stressed trees may flower and fruit out of season. A high incidence of wind thrown trees in a plantation is usually an indication of the presence of root-rot disease. When symptoms begin to appear in the aerial parts it is too late to save the tree. Fructifications of the fungus appear much later and often after the tree has been killed, and are therefore not helpful in the early diagnosis and control of the disease. To recognise the disease, one needs to look at the symptoms appearing on the roots.

Phellinus noxius causes a root-rot disease commonly known as brown root disease in which the roots are encrusted in a mass of earth, sand and stones intermingled and held together by rusty-brown velvety patches of mycelia. The fungus forms a continuous tawny brown fungal skin that darkens with age over infected roots and sometimes extends sock-like up the base of the tree. In the early stages, the rot is pale brown while in later stages brown zigzag lines appear in the wood which remains firm. When the rot is considerably advanced, the wood becomes friable, light and dry, permeated with sheets of brown fungal mycelia which form a honeycomb-like structure. The cells of the honeycomb may be hollow or filled with loose mycelia. A brown network of lines can be seen on the surface of the wood under the bark in advanced stages of the disease.

Phellinus noxius forms relatively small, hard fructifications, which may be pileate, effused-reflexed to resupinate. They may be solitary to imbricate. The surface of the pileus is at first finely velvety and pale ferruginous to umber in concentric zones, soon becoming glabrous in irregular sulcate zones and dark brown to black, covered with a 0.2–1 mm thick, resinous, hard crust. The margin is entire, round, often undulating and paler than the rest of the pileus. For further detailed descriptions, see Pegler and Waterston (1968) and Núñez and Ryvarden (2000).

Whether on roots or in heartwood, *Phellinus* rot can, with experience, be identified based on the characteristic pocket (honeycomb) pattern of the rotted wood.

While identification can be based on the presence of fruiting structures, these are seldom found. Instead, roots showing symptoms are collected and the fungi are isolated on artificial media and identified based on culture characteristics or after induction of fructification. For details of the technique, see Lee and Noraini Sikin Yahya (1999).

Step 3. Target host

The fungus is an important parasite on hardwood plantations in the tropics and although less frequent on conifers is a serious pest of hoop pine (*Araucaria cunninghamii*).

Step 4. Alternative hosts

None surveyed.

Step 7. The area

This survey can be applied to any hardwood plantation.

Steps 10 and 11. Site choice and sample size

Locations within a plantation where mortality due to root and/or butt rot disease has been observed in the previous crop/rotations should be targeted in subsequent surveys.

The number of plantations or field sites to visit may be determined by the range of plantations in the area of interest. Stands may be chosen according to such factors as age, provenance, soil type or because of the presence of dead trees.

The line transect method is recommended. A large-scale map of the stand should be created from an overview map of the area (1:5000 or better). Before conducting the ground survey, transects are laid out within the stand with the assistance of aerial photographs and walk-through reconnaissance data (when available).

A continuous line transect consisting of a strip between 2 and 5 m wide should be placed 50 m from the edge of the stand and not come closer than 10 m to any stand boundary. Parallel transects between 50 and 100 m apart are surveyed over the entire plantation. The length depends on the size of the blocks. Transect lines are flagged and marked for easy relocation and inspection.

Step 13. Data collected

The following information was collected along each transect line:

a. location of any dead or infected tree
b. status of tree (i.e. healthy, standing-infected, standing-dead, windthrown)
c. presence and extent along transect of infection centres.

Trees fallen due to windthrow were noted only if root disease could be identified as contributing to the cause of windthrow.

Root disease incidence can be calculated as follows:

$$\text{root disease incidence (\%)} = \frac{\text{total number of infected trees} \times 100}{\text{total number of trees examined}}$$

Step 14. Samples collected

Samples are collected when appropriate.

Step 12. Timing of survey

It is best to avoid conducting the survey during the dry season or if trees have shed their leaves (for species that defoliate seasonally) so as to prevent confusion of seasonal defoliation with defoliation due to root disease.

Comments

Various methods for the survey and assessment of root diseases can be found in the Root disease management guidebook, July 1995, authorised by the Forest Practices Code of British Columbia Act, Government of Canada. This can be found on the Internet at <http://www.gov.bc.ca/tasb/legsregs/fpc/fpcguide/root/chap3a.htm>.

References

Lee, S.S. and Noraini Sikin Yahya 1999. Fungi associated with heart rot of *Acacia mangium* trees in Peninsular Malaysia and East Kalimantan. Journal of Tropical Forest Science, 11, 240–254.

Núñez, M. and Ryvarden, L. 2000. East Asian Polypores. Vol. 1. Ganodermataceae and Hymenochaetaceae. Oslo, Norway, Fungiflora. Synopsis Fungorum 13.

Old, K.M., Lee, S.S. and Sharma, J.K., ed. 1997. Diseases of tropical acacias. Proceedings of an international workshop held at Subanjeriji (South Sumatra), 28 April–3 May 1996. CIFOR Special Publication, 53–61.

Pegler, D.N. and Waterston, J.M. 1968. *Phellinus noxius*. Commonwealth Mycological Institute Descriptions of Pathogenic Fungi and Bacteria No. 195.

8.16. Case study O. Monitoring survey of defoliation caused by a leaf disease in a plantation

Step 1. Purpose of the survey

This survey was used to measure the severity of damage (loss of functional leaf area) to a plantation after a leaf disease epidemic. The survey is appropriate for any type of crown damage caused by leaf pathogens or defoliating insects.

Step 2. Targeted pest names and diagnostic characteristics

Mycosphaerella leaf blight is caused by the pathogen *Mycosphaerella nubilosa.* This fungal pathogen infects the young, juvenile foliage of *Eucalyptus globulus* causing large blotchy lesions (Figure O1). The disease causes the soft, expanding leaves near the end of shoots to rapidly shrivel and detach producing a top-down pattern of defoliation (Figure O2).

While *M. nubilosa* is the main pathogen involved in the disease, a range of other *Mycosphaerella* species can be associated with the leaf lesions. The different species can be confidently identified and differentiated only by using DNA analysis.

Figure O1. Large blotchy leaf lesion on a juvenile leaf of *Eucalyptus globulus* due to infection by *Mycosphaerella nubilosa*

Figure O2. Top-down defoliation caused from the blighting infection by *Mycosphaerella nubilosa* of soft, young expanding leaves near the end of shoots

Step 3. Target host

Eucalyptus globulus

Step 4. Alternative hosts

No alternative hosts were surveyed.

Step 7. The area

The area was a *Eucalyptus globulus* plantation in northwestern Tasmania, Australia. The plantation was 2 years old and covered an area of 62 ha.

Steps 10 and 11. Site choice and sample size

We followed the method outlined in Stone et al. (2003) and divided the plantation into eight sub-areas to survey (Figure O3).

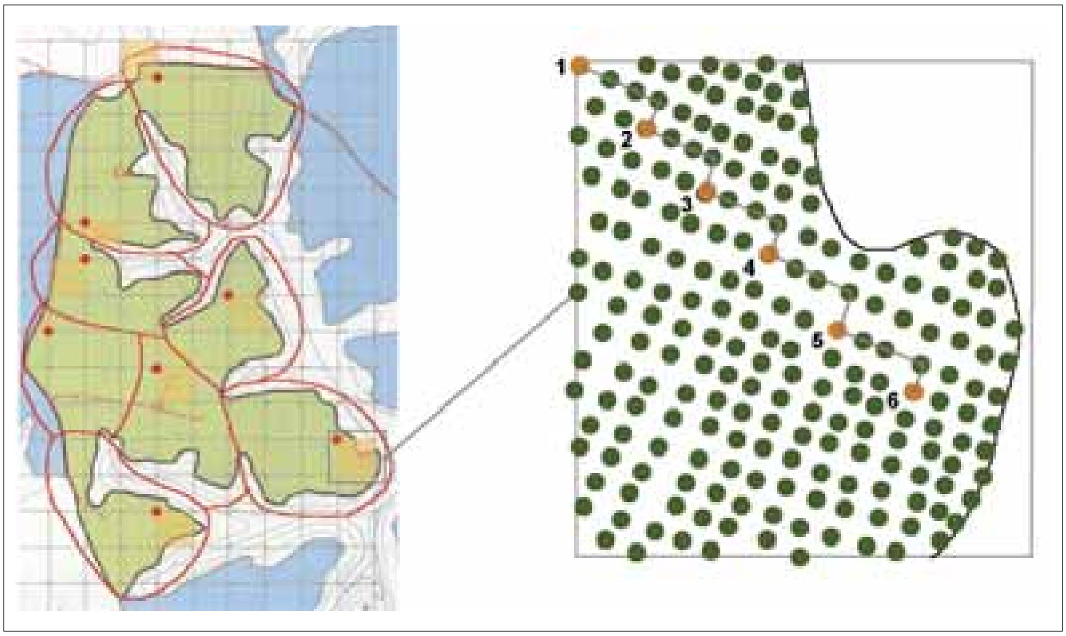


Figure O3. Map of the plantation to be surveyed showing how the plantation was sub-divided into eight sub-areas and the use of a 100 m × 100 m grid to randomly select a 1 ha cell within each of the eight sub-areas. The enlarged diagram on the right shows how six trees are selected for measurement from within the 1 ha cell using a stepped transect.

We drew a 1 × 1 cm grid on a map of the plantation (map scale equivalent to 100 m × 100 m on a 1:10,000 map). Each grid cell within each of the eight sub-areas was numbered (starting from the top left-hand grid cell in the sub-area) and a correspondingly numbered piece of paper was put into a jar. We then drew one number out of the jar to randomly select one of the grid cells within each of the eight sub-areas. (Figure O3). The top-left corner of the selected grid cell located the starting point of a stepped transect to select six trees to measure. Where the top-left corner fell outside the plantation, we progressed in a clockwise direction to the first corner of the grid that fell within the plantation. We walked to each of the selected grid corners, using the map for navigation. Once there, we found the tree closest to the corner and chose it as our first measurement tree. We then moved in a stepped transect (zigzag pattern) towards the diagonally opposite corner of the grid to select the remaining five measurement trees for that grid. The stepped transect involved moving three trees down the row from the tree just selected and then stepping across into the next row to select the closest tree (Figure O3).

Step 12. Timing of survey

The survey was carried out in late spring following a disease epidemic in late winter and early spring.

Step 13. Data collected

For each of the trees selected for measurement we estimated (i) the percentage of the tree crown that had been defoliated and (ii) the amount of leaf spotting in the remaining crown. These data are called crown damage index data. We estimated defoliation to the nearest 10% using a visual standard (Figure O4) to assist in making that estimate.

To estimate the amount of leaf spotting we estimated (i) the proportion of the remaining crown leaves with spots, and (ii) the average area of spots per leaf (Stone et al. 2003). The product of those two factors was converted to a percentage (by multiplying by 100) as shown below.

The total leaf area lost as a result of *Mycosphaerella* leaf blight was the sum of the defoliation estimate and the leaf-spotting estimate, e.g.

30% defoliation (i.e. 100 – 30% = 70% of leaves remained) [1]

50% of the leaves remaining had leaf spots [2]

the leaf spots affected an average of 30% of the leaf area [3]

Total leaf area lost = % defoliated + (leaves remaining % × 0.5 × 0.3)

Total leaf area lost = 30% + (0.5 × (100% – 30%) × 0.3) = 40.5%

We entered the field data into an Excel spreadsheet for calculating the crown damage index of a plantation. A reformatted version of this worksheet showing the data we recorded and the calculated value for average loss of leaf area ('CDI mean') is shown in Figure O5. A copy of the spreadsheet can be downloaded from the National Forest Inventory web site at <http://www.affa.gov.au/nfi>.

A selection of leaves with blotchy necrotic lesions was collected, bagged and returned to the laboratory for pressing and drying. The dried, pressed leaves were put into an appropriately labelled envelope (with accession number, collector, collection date, host species, location) and added to the disease herbarium if needed for future reference.

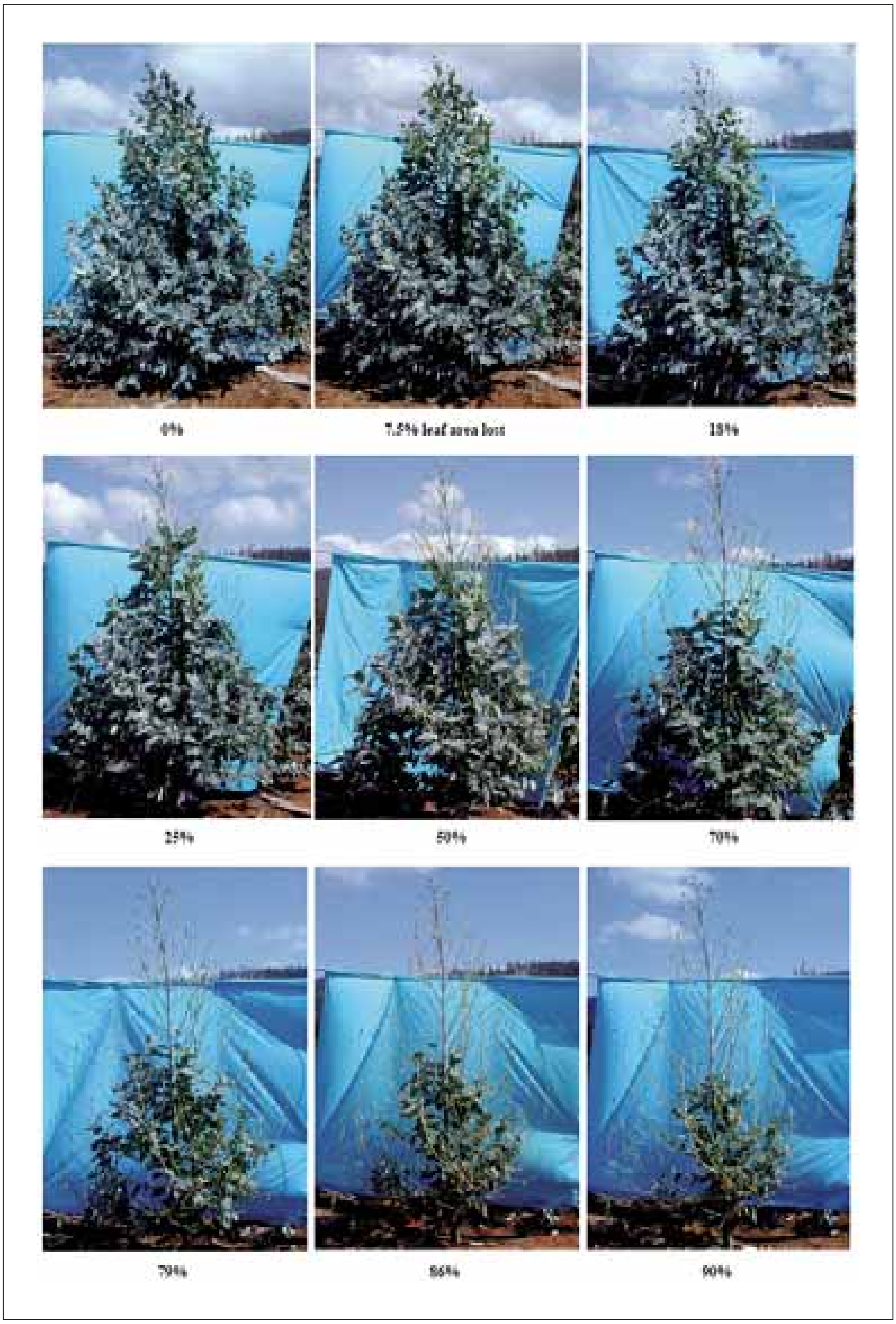


Figure O4. Visual standard for top-down defoliation of 2-year-old *Eucalyptus globulus* trees

date	10/23/01
assessor name	Tim Wardlaw
plantation name	NW Tas
year planted	1999
species	globulus
planted area for that species (ha)	62
size of grid cell (ha)	1
stocking rate (stems/grid cell)	1100
total number of grid cells	62
target 95%CI width (% of mean)	25

CDI	
mean	41
95%CI	6
target 95%CI	10
upper bound of 95%CI	47
lower bound of 95%CI	35
number of trees assessed	48

Defoliation	
mean	29.4
95%CI	5.2
upper bound of 95%CI	34.6
lower bound of 95%CI	24.2

Spots	
mean	11.8
95%CI	1.5
upper bound of 95%CI	13.3
lower bound of 95%CI	10.4

cell	tree	Defoliation	Spots	CDI	comments
1	1	40	15	55	
1	2	50	15	65	
1	3	20	20	40	
1	4	40	7	47	
1	5	30	12	42	
1	6	20	15	35	
2	1	10	7	17	
2	2	20	6	26	
2	3	20	12	32	
2	4	10	5	15	
2	5	30	12	42	
2	6	10	8	18	
3	1	30	12	42	
3	2	20	8	28	
3	3	30	10	40	
3	4	40	12	52	
3	5	20	12	32	
3	6	20	8	28	
4	1	60	8	68	
4	2	40	12	52	
4	3	20	12	32	
4	4	50	8	58	
4	5	40	10	50	
4	6	20	14	34	
5	1	20	16	36	
5	2	30	12	42	
5	3	30	15	45	
5	4	40	18	58	
5	5	20	12	32	
5	6	20	14	34	
6	1	30	12	42	
6	2	30	18	48	
6	3	10	12	22	
6	4	30	10	40	
6	5	60	12	72	
6	6	40	14	54	
7	1	30	12	42	
7	2	20	8	28	
7	3	20	6	26	
7	4	30	14	44	
7	5	10	12	22	
7	6	20	10	30	
7	10				
8	1	40	15	55	
8	2	50	16	66	
8	3	30	14	44	
8	4	20	12	32	
8	5	40	12	52	
8	6	50	12	62	

Figure O5. Completed spreadsheet showing the defoliation and leaf spotting measurements of 48 trees (six trees in each of eight sub-areas) and calculated values of crown damage index both for individual trees and overall for the whole plantation

Reference

Stone, C., Matsuki, M. and Carnegie, A. 2003. Pest and disease assessment in young eucalypt plantations: field manual for using the crown damage index. In: Parsons, M., ed., National Forest Inventory. Canberra, Australia, Bureau of Rural Sciences.

Comments

Equipment needed

One person is able to do this defoliation survey using:

- a map of the plantation drawn at an appropriate scale (e.g. 1: 10,000)
- visual standards appropriate for the type of damage being assessed (defoliation and leaf spots)
- a form for recording the crown damage index data.

For more information

Pest and disease assessment in young eucalypt plantations: field manual for using the crown damage index. September 2003. Australian Government Department of Agriculture, Fisheries and Forestry.

This handbook can be downloaded free from the Internet at:

<http://www.daff.gov.au/nfi>

and selecting 'Pest and disease assessment in plantations'. This page will also allow you to download a copy of the Excel spreadsheet that can be used.

8.17. Case study P. Survey to measure the incidence of trees with stem wounds

Step 1. Purpose of the survey

This survey was used to measure the prevalence of trees with stem damage. The survey is appropriate for any type of stem damage whether the cause is biotic (e.g. canker pathogens, stem boring insects), physical (e.g. fire) or mechanical (e.g. thinning wounds).

Step 2. Targeted pest name and diagnostic characteristics

Endothia gyrosa stem cankers were targeted. This fungal pathogen infects and kills the bark of a wide range of woody plants. *Endothia* can be recognised in the field by its small, black, fruiting bodies immersed within a pad of orange fungal tissue that erupts through the bark (Figure P1). The degree of damage caused by the pathogen can vary from negligible in the case of superficial cankers confined to the outer layers of bark (Figure P2) to extreme in the case of deep cankers (Figure P3) that can girdle the stem and kill the tree. In an outbreak, both superficial and deep cankers can occur.

Stem wounds are not easily seen, particularly if the forest has a dense understorey. Because of this, the survey needed to be ground-based to allow each tree in the sample to be closely inspected all the way around its stem.

Figure P1. Black fruiting bodies of *Endothia* immersed within a pad of orange fungal tissue that erupts through the bark

Figure P2. Superficial canker caused by *Endothia*: the infection has not penetrated the entire thickness of the bark so the cambium remains intact. This type of canker has little impact on stem quality

Figure P3. Deep canker caused by *Endothia*: the infection has penetrated the entire thickness of the bark and killed the underlying cambium. The bark within the canker splits and eventually sloughs off to reveal a stem wound.

Step 3. Targeted host

Shining gum (*Eucalyptus nitens*)

Step 4. Alternative hosts

No alternative hosts were surveyed.

Step 7. The area

The area surveyed was a *Eucalyptus nitens* plantation in northern Tasmania, Australia. The 25-ha plantation was 11 years old and had been recently thinned to leave behind a final crop of about 300 pruned trees for future harvest.

Steps 10 and 11. Site choice and sample size

One rectangular plot of 100 × 10 m was surveyed per 2 ha of forest. Once the number of plots required per coupe was calculated, plot locations were marked on a map of the coupe (1:10,000 scale was ideal). The plots were arranged at right angles from the previous plot without overlapping, to provide the best coverage of the coupe in a zigzag pattern (Figure P4). Whenever possible, the plots ran diagonally along the long axis of the coupe. We avoided locating the plots on log landings or other atypical areas.

A line 100 m long was marked out and trees with the middle of their trunk within 5 m of either side of the line were surveyed.

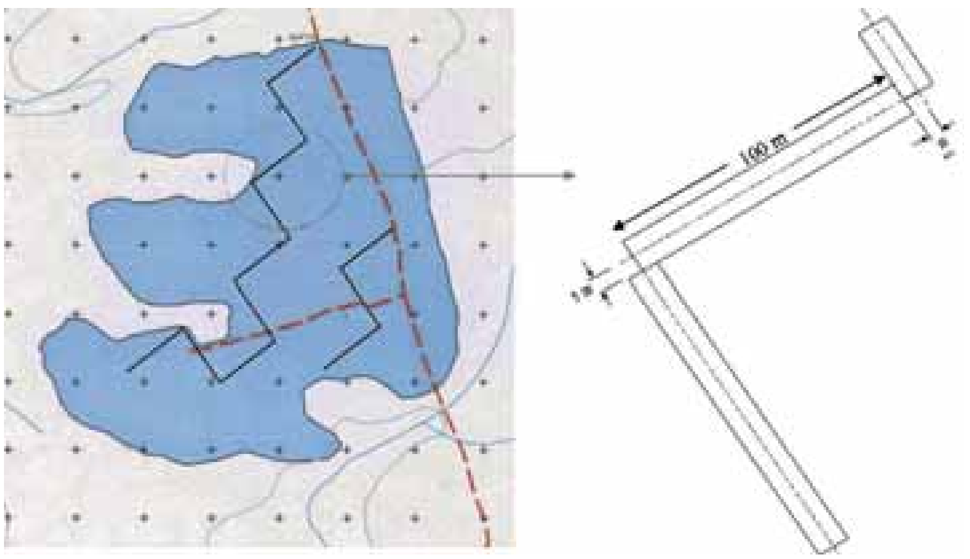


Figure P4. Zig-zag arrangement of 100 × 10 metre plots used for sampling stands to measure stem damage

Step 12. Timing of survey

The survey was performed in autumn soon after the damage was detected during routine health surveillance, but such a survey can be performed at any time of the year.

Step 13. Data collected

The survey was used to assess both superficial and deep cankers at the same time. A data sheet was used to record if each tree had superficial, deep or no cankers.

Data were collected for each plot separately (Figure P5). This permitted the total number of trees to be counted and the area covered was known. A percentage of trees per plot with each kind of canker was calculated. The data were used to calculate the average, standard deviation and 95% confidence interval for the data.

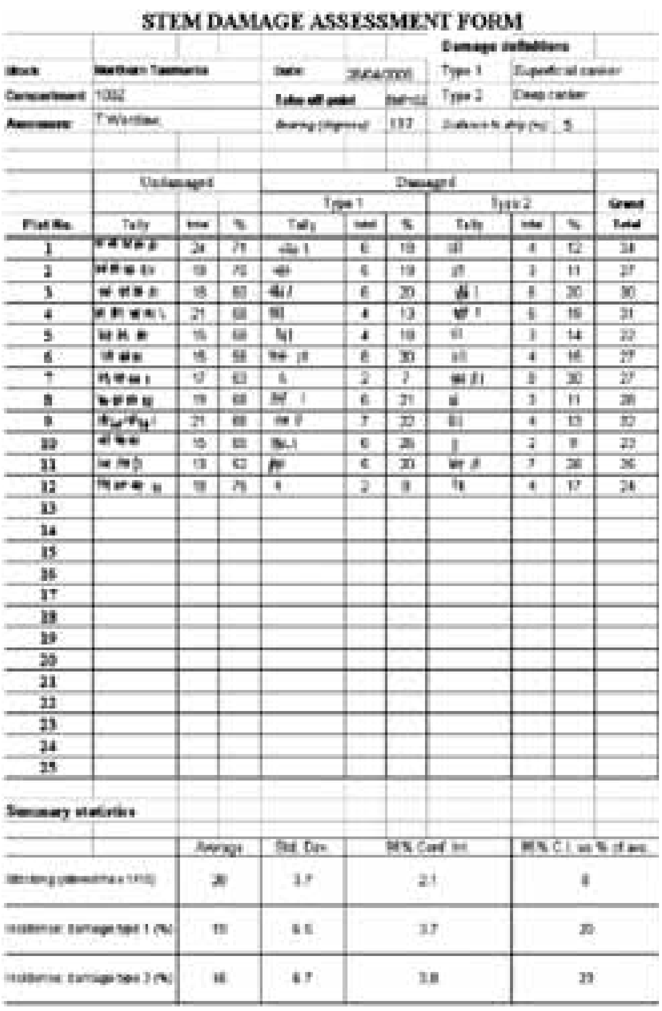
Step 14. Samples collected

The disease can be identified in the field with reasonable confidence solely on the basis of macro symptoms of the dark perithecia immersed in the orange fungal stroma (Figure P1). Nevertheless, several pieces of bark containing the fruiting bodies of the canker pathogen were collected using a hammer and chisel. These were returned to the laboratory, dried and stored with appropriate information (collector, collection date, host, location) as a record of the disease if required for future reference.

Comments

All plots were marked at each end and at the middle point, i.e. at 0, 50 and 100 m, using a coloured wooden peg or by marking a tree if at the correct distance.

Ensure there is sufficient room for the final 100 × 10 m plot. Only complete 100 m plots should be sampled. Where there is a chance that there will be insufficient room to complete a plot, do not survey it. Many errors have been perpetrated using half or parts of plots.


STEM DAMAGE ASSESSMENT FORM

Damage definitions: Type 1 – Superficial canker; Type 2 – Deep canker

	Undamaged			Damaged: Type 1			Damaged: Type 2			Grand
Plot No.	Tally	tree	%	Tally	total	%	Tally	total	%	Total
1		24	71		6	18		4	12	34
2		19	70		5	19		3	11	27
3		18	60		6	20		6	20	30
4		21	68		4	13		6	19	31
5		15	68		4	18		3	14	22
6		15	56		8	30		4	15	27
7		17	63		2	7		8	30	27
8		19	68		6	21		3	11	28
9		21	66		7	22		4	13	32
10		15	65		6	26		2	9	23
11		13	[illegible]		6	[illegible]		7	[illegible]	26
12		18	75		2	8		4	17	24
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										

Summary statistics

	Average	Std. Dev.	95% Conf. Int.	95% C.I. as % of ave.
[illegible]	28	3.7	2.1	8
Incidence damage type 1 (%)	19	6.5	3.7	20
Incidence damage type 2 (%)	16	6.7	3.8	23

Figure P5. An example showing how tree tallies are recorded and calculations made in the damage assessment form

Equipment needed

A team of at least two people is required to properly do a damage survey. They will need the following equipment:

- a compass to ensure successive plots run at 90° to one another
- a 50 m measuring tape to measure two 50 m lengths
- a 10 m measuring tape to check the plot width
- a damage assessment form
- a calculator
- a hammer, chisel and paper bag to collect and store samples of the canker.

Reference

Wardlaw, T.J. 1999. *Endothia gyrosa* associated with severe stem cankers on plantation grown *Eucalyptus nitens* in Tasmania, Australia. European Journal of Forestry Pathology, 29, 199–208.

8.18. Case study Q. Monitoring survey in pine plantations

Step 1. Purpose of the survey

This survey was used to monitor the presence of pests or diseases in plantations at a level where the resultant damage is probably sufficient to require remedial treatments. The survey is appropriate for detecting pests and diseases that produce obvious symptoms, such as mortality, dead tops and significant defoliation. Pests and diseases that produce symptoms confined to the stem, such as canker pathogens or stem-boring insects, cannot be reliably detected using this method unless damage is severe enough to result in tree death. Ground surveys are needed to detect pests and diseases that cause such cryptic symptoms.

Step 2. Targeted pest names and diagnostic characteristics

Usually the target pest will not be known and is not normally encountered. However, early detection surveys will often be an important part of the management of pests that are uncommon but can build to damaging populations very quickly. *Sirex noctilio* (Sirex) wood wasp is one such pest. This stem-boring insect lays its eggs in the sapwood of *Pinus* spp. When the wasp lays its eggs it also secretes a toxic mucus and introduces the fungus *Amylostereum aureolatum*. The combination of the mucus and the fungus causes heavily attacked trees to wilt and die. Trees killed by Sirex have beads of resin flowing down the trunk from the egg-laying sites (Figure Q1). If the adult wasps have emerged, the trunk will also have circular exit holes about 5 mm in diameter (Figure Q2). The wasp can very quickly (over 2–3 years) build up to large populations causing widespread tree losses.

Figure Q1. Pine tree showing beads of resin flowing from egg-laying sites of the wood wasp, *Sirex noctilio*

Figure Q2. Pine tree showing circular exit holes where adult Sirex wasps have emerged

Step 3. Target host

Pine tree (*Pinus radiata*)

Step 4. Alternative hosts

No alternative hosts were surveyed.

Step 7. The area

The area was a *Pinus radiata* plantation estate in northern Tasmania, Australia.

Steps 10 and 11. Site choice and sample size

Within the estate, the focus for Sirex detection was on densely stocked plantations approaching mid-rotation (10–15 years old) on drier sites. These plantations are most susceptible to attack by Sirex

Because of their high density, plantations that are most susceptible to Sirex attack are very difficult to inspect from the roadside or the ground. An overview inspection from the air (Figure Q3) or a high vantage-point (Figure Q4) is the best way to look for Sirex-killed trees. Aerial inspection uses either a helicopter or fixed-wing plane flying at an altitude of 150–200 m above the ground and at speeds of no more than 180 km/h. Vantage-point inspection involves driving or walking to high vantage-points such as a hill top or fire tower and systematically viewing the plantation either with the naked eye or using binoculars. The entire target area needs to be inspected regardless of the method used (air or vantage-point). Where an overview inspection is not possible (this includes areas that cannot be seen from vantage-points) an intensive ground survey needs to be done. This involves walking up or down every third row and inspecting the crowns of individual trees. If recently dead or dying trees are detected during the overview inspection, their location needs to be mapped for follow-up inspection from the ground to confirm the cause of death and, in particular, whether or not Sirex is present.

Static traps using α-pinene as an attractant (Figure Q5) can be used in susceptible plantations as an alternative to overview inspection or ground surveys for detection of Sirex. Static traps are able to detect Sirex at very low populations. However, they do require regular (fortnightly) servicing during the trapping period.

Step 12. Timing of survey

This survey was performed in spring, but the work can be done at any time of the year. Response actions to a detection of Sirex need to commence by late autumn to early winter. Because of this, detection surveys targeting Sirex are usually carried out between spring and early autumn.

Step 13. Data collected

The locations of confirmed Sirex-killed trees were recorded. The locations were recorded either as an annotation on a map or as grid co-ordinates obtained using a GPS instrument.

Figure Q3. Tree death caused by *Sirex noctilio* wood wasp can be visible from small plane or high vantage-point

Figure Q4. Example of hilly terrain where trees could be surveyed for damage from a high vantage-point

Figure Q5. Static traps using α-pinene as an attractant for *Sirex noctilio* wood wasp

Equipment needed

A team of two people is needed to properly do a detection survey.

Accurate maps of the plantations being inspected are vital. Maps with a scale of between 1:10,000 and 1:25,000 are most useful for detection surveys. Larger scale maps with scales of between 1:100,000 and 1:250,000 are useful for navigating if you are inspecting the plantations from an aircraft or helicopter. Maps showing contours, water-courses, roads and forest compartments are most useful for mapping the location of trees with symptoms detected from the air or vantage-points.

A compass, protractor and ruler are useful to locate affected trees detected from vantage-points (obtain a compass bearing from the vantage-point to the tree of interest and draw a line of that bearing on the map using a protractor and ruler).

A GPS instrument is useful for obtaining an accurate location of affected trees.

Binoculars are useful for vantage-point inspections of plantations. They allow you to inspect the crowns of individual trees.

8.19. Case study R. Monitoring survey of aphids on crucifers

Step 1. Purpose of the survey

This survey in Vietnam was performed to establish which aphids were present on cruciferous crops in different provinces and which crops they preferred as hosts.

Step 2. Targeted pest names and diagnostic characteristics

The four aphid species that have cruciferous hosts in Vietnam are:

- *Aphis craccivora* (Koch)—apterae small, adults shiny black. This species is sometimes found between April and July in small colonies on crucifers.
- *Aphis gossypii* (Glover)—apterae very variable in colour on many crops, but on crucifers the adults are dark green and found early in the season (October–November), in small colonies.
- *Brevicoryne brassicae* (L.)—apterae of medium size, 1.5–2.5 mm, greyish green with dark head and dark dorsal thoracic and abdominal markings. The body is covered with greyish white mealy wax which is also secreted onto the surface of host plants. Alatae are 1.3–2.4 mm, with a dark head and thorax and black transverse bars on the dorsal side of the abdomen.
- *Myzus persicae* (Sulzer)—apterae small to medium size, 1.2–2.1 mm, whitish green, pale yellow-green, grey-green, mid-green, pink or red. Alatae have a central black patch on the dorsal side of the abdomen; immatures of the alate morph, especially in autumn populations, are often pink or red.

Brevicoryne brassicae and *M. persicae* are typically the dominant aphids. The life-span of *B. brassicae* is 5–10 days, and one apterous female can produce 19–33 nymphs. The life span of *M. persicae* is 6–13 days and females can produce 25–60 nymphs.

In most cases, aphids affect the tender, growing parts of plants. Large colonies can form under the leaves and also on the flower heads of seed crops. When plants are heavily affected, host symptoms are curling of tender leaves, twisting of the tender shoots, yellowing of foliage and stunted growth.

Identification of aphids was confirmed by a taxonomist.

Step 3. Target host

Brevicoryne brassicae (L.) lives on most cruciferous plants, which are planted every month of the year in many provinces. The targeted plants included cabbage, brussels sprouts, radish and cauliflower.

Myzus persicae (Sulz.) also lives on most cruciferous plants and tobacco, from October to June in the Red River Delta region. In summer (June–September) we surveyed Cruciferaceae in only northern mountain areas.

Step 4. Alternative hosts

Brevicoryne brassicae was also surveyed on grape and *M. persicae* on tobacco, peach, pawpaw, water chickweed (*Myosoton aquaticum*), citrus and water spinach (*Ipomoea aquatica* Fosk-Laportea).

Step 7. The area

The areas surveyed were the largest crucifer production areas in Vietnam. These were suburbs of Hanoi, Haiphong and Sapa in the north of the country and Dalat in the south. These areas have diverse topography, soil conditions, seasonal patterns and host varieties.

Steps 10 and 11. Site choice and sample size

The places were production areas. Field sites were defined as crop fields.

Due to the limited time, we surveyed 3–5 representative fields of the main seasonal crops and alternative hosts (about 27 fields). Fields were visited five times at 5-day intervals.

We surveyed five sampling sites in each field. Within each sampling site, we selected 10–12 plant parts (shoots, flowers, young plants) that belong to one of five arbitrary classes of infestation (10–12 very light leaves, 10–12 light leaves, 10 medium, 10 heavy). See below.

Step 12. Timing of survey

Survey was carried out at 5-day intervals because the shortest life span of the aphids is approximately 6 days.

Step 13. Data collected

At each site, aphids were counted on 5–10 plants or a 20 cm^2 area for seedlings.

Aphids on the plant parts (leaves, stems, shoots, flowers or whole seedlings) were ranked into one of five arbitrary classes of infestation:

zero:

no aphids seen

very light:

from one aphid to a small colony on the leaves

light:

several aphid colonies on the leaves

medium:

aphids present in large numbers, not recognisable colonies but diffusing and infesting a large proportion of the leaves and stem

heavy:

aphids present in large numbers, very dense, infesting all the leaves and stems.

The main data recorded were:

- number of aphids per leaf, shoot, flower, stem or seedling
- number of plant parts showing aphid symptoms per field
- number of natural enemies of aphids observed
- phenology of crop plants
- daily weather conditions.

Entire plants were pressed and photographs were taken.

Data sheets were used to record the data, which were then transferred to Microsoft Excel data sheets.

Step 14. Samples collected

Twelve sample leaves in each class were collected and aphids from each leaf were collected in 90% alcohol in sealed containers to be later counted in a laboratory. Counts per leaf were recorded.

8.20. Case study S. Monitoring survey for phosphine resistant stored grain insects

Step 1. Purpose of the survey

To monitor any grain insects resistant to the fumigant, phosphine.

Step 2. Targeted pest names and diagnostic characteristics

Targeted were any phosphine resistant grain insects including beetles, weevils and moth larvae. All grain beetles are small, 2–5 mm long and brown to black. True weevils (a type of beetle) are identified by their elongated snout. The larvae of the grain moths are generally pinkish or cream and may produce webs. Those species that are able to attack whole grains and cause primary damage are the more economically important and include:

- lesser grain borer (*Rhyzopertha dominica* (F.))—Figure S1
- rice weevil (*Sitophilus oryzae* (L.))—Figure S2
- granary weevil (*Sitophilus granarius* (L.)).

The other species cannot attack whole grain but cause secondary damage by eating cracked and broken grains which are found with whole grain. These include:

- rust-red flour beetle (*Tribolium castaneum* (Herbst))—Figure S3
- confused flour beetle (*Tribolium confusum* Jacquelin du Val)
- sawtoothed grain beetle (*Oryzaephilus surinamensis* (L.))—Figure S4
- flat grain beetles (*Cryptolestes* spp.)—Figure S5
- some species of booklice (order Psocoptera).

Figure S1. Lesser grain borer (*Rhyzopertha dominica*)

Figure S2. Rice weevil (*Sitophilus oryzae*)

Figure S3. Rust-red flour beetle (*Tribolium castaneum*)

Figure S4. Sawtoothed grain beetle (*Oryzaephilus surinamensis*)

Figure S5. Flat grain beetles (*Cryptolestes* spp.)

The 'rapid test' (Reichmuth 1991), was used to give a quick resistant/not resistant (+/–) result with field collected insects, allowing immediate action (control, eradication or quarantine) to be taken where appropriate.

To more accurately assess insects found to be resistant, one or other of two assays was used.

The first bioassay used the standard FAO technique of placing insects into gastight desiccators and adding phosphine (FAO 1975). Two discriminating doses were used. A lower one discriminated between susceptible and resistant insects and a higher one is designed to detect resistances higher than the common 'weak' resistance (Daglish and Collins 1999). The discriminating doses listed in the original method have been adapted according to responses of Australian laboratory reference strains. Insects believed to be homozygous for phosphine susceptibility have been used to determine the lower discriminating dose, while strains homozygous for weak resistance were used to determine the upper discriminating dose. Susceptibility was determined by the dose at which the insects died.

The other assay method used was the flow-through technique that exposes mixed-age cultures of insects to a continuous flow of phosphine at a constant concentration (Winks and Hyne 1997; Daglish et al. 2002). This method is very laborious and lengthy but it gives an accurate prediction of the time required for complete extinction of an insect population at a given phosphine concentration (Daglish and Collins 1999). It is used to characterise the resistance and predict concentrations and exposure periods needed to control insects in the field.

Step 3. Target host

Grain, cereals and products including wheat, barley, oats, rye, maize, rice, flour, malt, and noodles.

Step 4. Alternative hosts

None were surveyed.

Steps 7, 8 and 9. The area, place, district and field sites

The survey targeted grain-export terminals, freight terminals, farm storages, bulk-handling companies and grain processors across Australia, where the grain insects are known to be present or at risk of infesting. Other sources included household samples and quarantine interceptions which could be a source of resistant strains from outside the country.

Steps 10 and 11. Site choice and sample size

Sampling sites were targeted during routine inspection of all the field sites and locations listed at Step 7. Staff targeted field sites where the practices were not hygienic or where it was suspected that insects might be resistant to phosphine.

Step 12. Timing of survey

Inspections were performed during the warmer summer months, when beetle activity is at it highest (October–April). In warmer climates, the beetles can survive all year round and so continuous surveillance is required. At terminals, the trapping was continuous.

Step 13. Data collected

Collector, date, location (including latitude/longitude), property name, property type, owner, survey type, commodity infestation level, and comments including sample position within the storage site were recorded. Following resistance assays, the test date, dose, actual dose, exposure period, number of insects tested and the number surviving the discriminating dose.

Step 14. Samples collected

Insects were collected using a grain sieve. The recommendation was that at least 100 live insects of each species be collected from each site for the assays.

Pheromone and probe traps were of limited use as the insects were often dead by the time they were returned to the laboratory.

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FAO (Food and Agriculture Organization) 1975. Recommended methods for detection and measurement of resistance of agricultural pests to pesticides. Tentative method for adults of some major pests of stored cereals, with methyl bromide and phosphine. FAO Method No. 16. Rome, FAO Plant Protection Bulletin, 23, 12–26.

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8.21. Case study T. Papaya infecting strain of papaya ringspot virus (PRSV-P): a delimiting survey

Step 1. Purpose of the survey

To determine whether an outbreak of papaya ringspot virus in a single papaya tree on the island of Rarotonga was an isolated occurrence or indicated more widespread infestation. This followed confirmation of PRSV infection in leaf samples sent to Fiji and Australia.

Papaya growers and staff of the Ministry of Agriculture, Cook Islands, were on the alert for symptoms of this exotic disease. It had recently been confirmed in neighbouring French Polynesia and SPC had distributed a PestAlert (a one-page information flyer with colour photographs).

Step 2. Targeted pest name/s and diagnostic characteristics

The principal symptom of papaya ringspot virus is a strong yellow, mosaic and mottling pattern on leaves that is visible, when advanced, from a long distance. Other leaf symptoms are blistering, distortion and sometimes a 'shoestring' symptom (leaf lamina reduction). Symptoms on fruits are characteristic to only this disease. These are dark-green on light-green target-like rings, spots and C-shaped markings which become dark orange-brown as the fruit ripens.

Diagnostic testing in Fiji was performed by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and the back-up confirmation in Australia was performed using reverse transcription polymerase chain reaction (RT-PCR).

Step 3. Target host

Carica papaya (papaya, pawpaw)

Step 4. Alternative hosts

None surveyed.

Step 7. The area

Rarotonga is an island 32 km in circumference, with a rugged interior of mountains (highest point is 658 m) covered in native bush, surrounded by a narrow band of agricultural land. This includes many large and small commercial plantings of papaya (cv. Waimanalo), grown for export to New Zealand and also for the local market (the value of annual production exceeded NZ$1 million in 2004). Throughout the cultivable area, papaya trees grow in many domestic and tourist industry properties.

The temperature averages 18–28°C in winter and 21–29°C in summer.

Steps 10 and 11. Site choice and sample size

The sites were chosen based on where the pest was found and by the anticipated rate of spread. Aphid-borne movement of virus would be limited in two directions as flights in one would meet the forested interior (where aphids would lose a non-persistent virus when feeding on non-hosts) and in another would meet the sea. Spread by humans (via infected seedlings) is possible to anywhere.

The sites surveyed were:

1. the closest 55 trees to the single diseased tree
2. the remainder of the 300 trees in the plot that contained the original diseased tree plus four adjacent plantings
3. all commercial and domestic plantings within 2 km of the diseased tree
4. every other known commercial plot.

Over 5000 trees were individually surveyed and thousands more were observed from various distances. Observations were made by walking every second to fifth row, depending on size of the plantations.

Step 12. Timing of survey

The survey took place 5–6 weeks after the outbreak was discovered. This was to ensure detection of any natural spread that may have occurred from the original infected tree to other trees before its death. Additionally, symptoms usually take 3–4 weeks to appear in the field following aphid transmission.

Step 14. Samples collected

Time available for laboratory testing limited the number of leaf samples that could be collected. Apart from sub-area 1, leaf samples were collected because they were showing various leaf abnormalities slightly similar in some ways to those caused by the viral disease.

The 281 leaf samples collected comprised:

1. one from each of the 55 nearest trees, regardless of the appearance of their leaves
2. 16 samples from the affected planting (tree plot) and 15 from the four nearest plantings
3. 83 from plantings or domestic compounds within a 2 km radius of the first detection
4. 112 from commercial plots elsewhere.

Fresh leaf samples were collected, symptoms noted, then stored at 4°C before testing (up to 8 days later) by DAS-ELISA at Totokoitu Research Station. The plus or minus test result threshold used was absorbance readings of greater than three times the mean of four negative control leaves included on each test plate.

Comments

The eradication was successful because of a rapid response by the government and laboratories involved and by the early detection of the pest which was spotted by a grower who was aware of what to look for thanks to the SPC publicity.

8.22. Case study U. Delimiting survey for Huanglongbing disease of citrus and its vector the Asian citrus psyllid in Papua New Guinea

Step 1. Purpose of the survey

The purpose was to perform a delimiting survey following the detection of Huanglongbing in Vanimo, Papua New Guinea. In the initial detection, 1 of 20 trees examined during a plant health survey had tested positive.

Step 2. Targeted pest name and diagnostic characteristics

Huanglongbing (‘*Candidatus* Liberibacter asiaticus’) is an unculturable, phloem-limited bacterium that is vectored by the Asian citrus psyllid, *Diaphorina citri*. The disease caused by Huanglongbing (HLB) is also known as citrus greening disease.

Diagnosis of HLB is difficult because the symptoms are the same as for deficiencies of nutrients such as zinc and manganese, and similar to other disorders. Confirmation of HLB is performed by DNA based PCR tests of leaf tissues from trees that are showing possible HLB symptoms. A yellowing of one section of the tree is the clearest indication of early stages of infection. Leaves display interveinal chlorosis, leaf size is reduced and leaf growth tends to be more upright. Chlorotic blotching, together with one or more other leaf or branch symptoms, especially if accompanied by noticeably swollen veins, may also be a sign of infection. Chronically infected trees are sparsely foliated and stunted, with leaves almost completely devoid of chlorophyll. Fruit may have a lopsided shape with a curved columella.

The Asian citrus psyllid has a high fecundity and a short life cycle (around 14 days) in the absence of natural controls. Eggs are approximately 0.3 mm long, almond shaped, thicker at the base and laid on new shoots. Freshly laid eggs are light yellow but turn bright orange with two distinct red eye spots at maturity. There are five nymphal stages, ranging from 0.25 mm to 1.7 mm. Nymphs have a light pink body and a pair of red compound eyes. In some mature nymphs, the abdomen turns bluish-green instead of pale pink. Adult psyllids can live for 6 months and are 3–4 mm long with a yellow-brown body and greybrown legs. Wings are transparent with a broad light-brown marginal band distally on the fore wings. Adults are often found resting on the terminal portion of plant, especially on the lower side of the leaves with their heads pointing downward to the leaf surface at an angle of 30°. When disturbed they readily take flight for a short distance.

Diaphorina citri stunts and twists young shoots, so that the growing tips present a rosetted appearance. Leaves are badly curled, and may be covered with honeydew and sooty mould. Leaves may drop prematurely.

Step 3. Target hosts

Targeted hosts for HLB and the psyllid were all citrus plants. There are different susceptibilities amongst citrus species. HLB symptoms are most severe on mandarins, sweet oranges and their hybrids, moderate on grapefruit, lemon and sour orange, with weak symptoms evident on lime and pomelo.

Step 4. Alternative hosts

None were surveyed.

Step 7. The area

The initial infection was detected in Vanimo, in the Sandaun Province of PNG. The town of Vanimo is in a remote area and has a population of about 10,000 (PNG National Census data, 2000). All villages with road access or frequent boat contact with the site of the first detection, Vanimo, were also surveyed.

Survey 1 was conducted in and near Wewak, East Sepik Province and in and near Vanimo, Sandaun Province, totalling 12 villages and including 2 towns. In Survey 2, the same places were surveyed plus coastal villages that have regular contact with Vanimo (both east and west of Wewak as far as Aitape and nearby villages). Survey 2 also included villages inland from Vanimo up to the Bewani region, totalling 23 villages and including 3 towns.

Steps 10 and 11. Site choice and sample size

In Survey 1, within Vanimo, approximately one tree in every third private backyard in the surrounding streets was examined. Around Wewak the survey was less intensive.

In Survey 2, the streets around the initial infection site were heavily targeted so that all accessible trees were surveyed. At the remaining villages, the most suspicious looking tree was surveyed.

Seventy-two trees were surveyed in the first survey and 48 in the second survey.

Step 12. Timing of survey

The initial detection of the disease arose from an identification of the psyllid, which prompted an extensive collection of citrus leaves for testing. The first follow-up survey was conducted as soon as it could be arranged (2 months after detection in November 2002), to determine the extent of the disease infestation. The second follow-up survey was conducted 12 months later in November 2003.

Psyllid numbers can fluctuate throughout the year, depending on rainfall and when new growth appears on citrus hosts. The surveys were conducted in November for two reasons; November was still relatively dry, which was important because periods of rain (as often follows from December to April) depress psyllid populations, and the new active leaf growth had begun.

Step 13. Data collected

The following data were recorded for all samples: sample identification number, date of collection, country, a description of the location—e.g. person's house, street number or nearest major town, GPS coordinates, plant type and name, and name of collector.

Step 14. Samples collected

Citrus trees showing possible HLB-like symptoms were sampled. Samples consisted of 10–20 leaves per tree, prepared according to the method outlined below.

To sample for psyllids, new growth flushes of host plants were examined for the presence of adults or nymphs. Where psyllids were observed, trees were sampled by sweep net and psyllids collected from the net using an aspirator or pooter. Nymphs were collected using either fine forceps, a scalpel blade or paint brush. Psyllids were then stored in glass vials containing 70% alcohol.

Comments

Collection and desiccation technique of leaves for Huanglongbing identification

- Collect 10 to 20 leaves displaying symptoms (aim for 1–2 g fresh weight of excised petioles and midribs). Number is dependent upon leaf size. Smaller leaves will require a larger number to be collected.
- If possible, surface sterilise leaves with 70% alcohol or 1% pool chlorine.
- Using a sharp knife cut midribs and/or petioles from leaves and chop midribs and petioles into approximately 2–3 mm lengths (Figure U1). The disease organism is limited to the midribs and petioles of the plant. It is important to collect only this material as any extra leaf material may reduce the sensitivity of the test
- Wrap in paper (facial) tissue or medical gauze bandage and place over calcium chloride in 25 mL plastic vials, wrap Parafilm or insulation tape around the join of the lid and vial and place it immediately in the refrigerator. Calcium chloride will dry the leaf material so that it can be sent for testing.
- The following day replace the paper tissue or gauze with fresh, dry paper tissue or gauze and reseal vials. If possible store in the refrigerator or in a cool box with ice. For long-term storage, material must be held in a refrigerator.
- If sending material to a country free of HLB for testing, ensure quarantine import permits are in place before consigning the samples. Sample vials must be consigned in a robust screw-cap container.

Figure U1. Preparation of leaves for later plant pathogen identification

8.23. Case study V. Delimiting survey for red-banded mango caterpillar in northern Queensland

Step 1. Purpose of the survey

Delimiting surveys for red-banded mango caterpillar (RBMC, *Deanolis sublimbalis*) help the Queensland Department of Primary Industries and Fisheries (QDPI&F) formulate risk-management options that reduce the potential impact upon commercial mango production areas to the south. The survey also involves a general surveillance component of public awareness and reporting campaigns.

Step 2. Targeted pest name/s and diagnostic characteristics

RBMC has slowly spread from Papua New Guinea through the Torres Strait Islands and was first detected on mainland Australia in 2001. The pest is under active control, with regulations prohibiting movement of mango fruit or plants on northern Cape York Peninsula.

Caterpillars burrow into the mango fruit causing severe damage and fruit drop. Holes in fruit with weeping sap stains are a good indicator of pest presence. The caterpillar is distinctively coloured and easy to identify when the fruit and seed are cut. Samples are taken to compare to existing reference collection material and to substantiate pest records. Identification can be confirmed by DNA sequencing at the Australian National Insect Collection.

Step 3. Target host

RBMC host range is restricted to mangoes (*Mangifera* spp. and *Bouea* spp.).

Step 4. Alternative hosts

None.

Step 7. The area

The survey area covers Cape York Peninsula in northern Queensland, commercial production areas around the Atherton Tablelands near Cairns and the regional cities of Cairns, Townsville and Mackay (Figure V1).

The current infested area of feral mango trees is in rainforest in the northernmost 30 km of Cape York Peninsula known as the Northern Peninsula Area. Scattered mango trees near old sites of human activity make a bridge to the Northern Peninsula Area Indigenous communities where hundreds of mango trees are present. RBMC is currently separated from commercial mango production areas by 700 km of sparsely populated, inhospitable habitat so the main threat comes from caterpillars being carried in fruit.

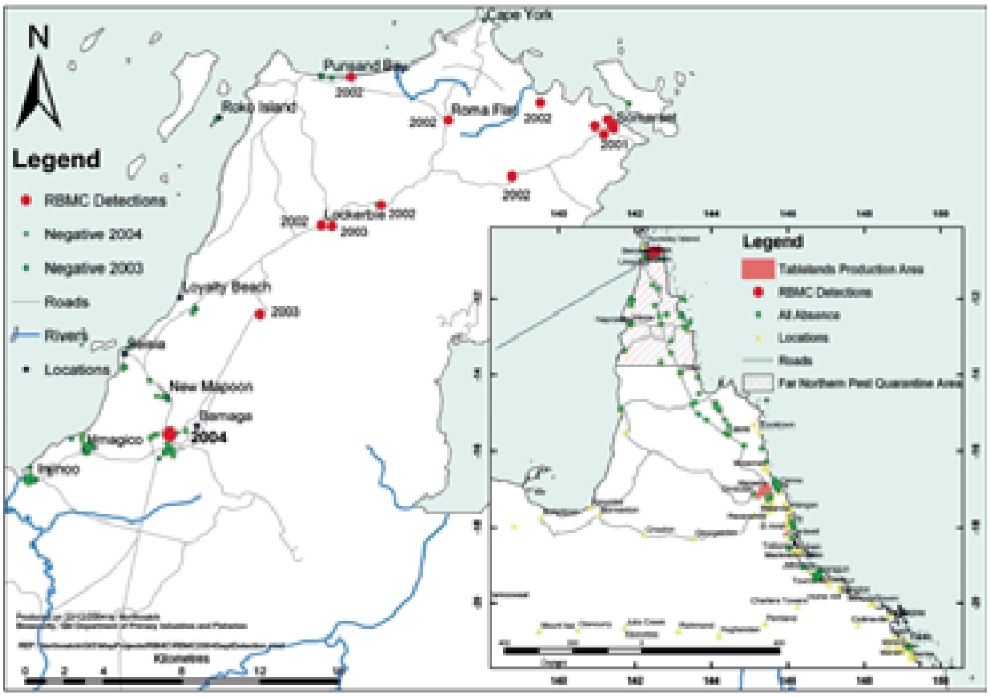

Figure V1. QDPI&F delimiting surveillance for RBMC

Steps 10 and 11. Site choice and sample size

Surveys targeted the pathways that the pest might use to reach production areas. Early delimiting surveillance was used to support an eradication attempt. After the eradication area was breached, the control options concentrated on regulatory restrictions on fruit movement and public awareness campaigns.

The most immediate pathway is natural spread through the Northern Peninsula Area communities, followed by movement in fruit to tourist sites, communities on Cape York Peninsula, major regional cities and production area towns.

Range expansion from natural spread is delimited by examining all known trees around the perimeter of the infested area annually. The Northern Peninsula Area communities are exposed to both the threat of natural spread and the threat of illegal movement of infested fruit. Approximately one quarter of all trees in the communities are examined and at least 10 of the most suspicious fruit per tree are cut. This surveillance intensity will detect RBMC before populations reach a size that imposes a significant additional risk of transport from the communities towards production areas.

As far as possible, a uniform coverage for each community is surveyed to increase the chance of detecting an incursion. Random sampling is too time-consuming to implement and can result in larger areas not being inspected and adjacent areas that are likely to have similar fauna being over-examined.

NAQS surveillance and regulatory activity target air and sea pathways for RBMC which reduces the delimiting surveillance activity required of the QDPI&F. A single road pathway connects the infested area to the production area. All mango trees at tourist stops on this road are examined annually with additional trees examined around the few towns. Surveillance of fruit is conducted at a quarantine control point at which all mangoes are confiscated from travellers.

Around production areas, roadside and backyard trees are examined as they are not treated with insecticides and are more likely to be in areas exposed to discarded, infested fruit. Public awareness material distributed to growers is the most effective way of monitoring for the pest on farms. Annual surveys in these areas give confidence to growers that the pest is not common in the immediate vicinity and is unlikely to affect them in the season.

Year	Sites	Trees at sites	Trees examined	Fruit cut
2001	240	1,050	898	657
2002	98	999	746	770
2003	129	1,128	647	293
2004	48	357	351	2,701
Total	515	3,534	2,642	4,421

Surveillance of a wide range of pests in urban areas (see also Case study D) targets the spectrum of potential exotic pests. As most of the work involved in surveillance is in getting to sites, gardens that have a broad host range are targeted as they could support high-priority pests. RBMC is one of these pests and information on its absence is collected if fruit is available to be cut.

Public awareness campaigns encourage the public to report the pest, which augments confidence in pest absence records. Material asking people to report the pests is specifically designed for Indigenous communities, tourists, growers and urban residents. Extensive signage informing people of the regulatory restrictions is deployed along paths where the pest is likely to move.

Step 12. Timing of survey

Surveys around the infested area are timed to coincide with good fruit development, but early in the season before the roads become impassable. Late season surveys are conducted around production areas, while urban area surveys are staggered throughout the year for different pests.

Step 13. Data collected

Data recorded for each site are the observers' names, date, site description, GPS location, number of trees present, number of trees examined and number of fruit cut. Absence data are recorded explicitly on the form and suspect samples are collected into ethanol.

Comments

Knowing where the pest is helps authorities to distribute public awareness material to the right place, modify internal quarantine inspection so as to mitigate the risk of fruit movement and keeps commercial growers informed of the level of threat to their industry.

8.24. Case Study W. Delimiting survey of the Queensland fruit fly in Rarotonga, Cook Islands

Step 1. Purpose of the survey

Queensland fruit fly, *Bactrocera tryoni* (Froggatt), was detected at the Punanga Nui market in Avarua, Rarotonga, Cook Islands on 21 November 2001, 500 m from the wharf. This survey was part of an emergency response and eradication program.

Step 2. Targeted pest name and diagnostic characteristics

Bactrocera tryoni is considered to be a fruit fly exotic to the Cook Islands. It is about 7 mm long, almost the same size as a housefly. It is distinguished from two other species present in the Cook Islands by the predominantly red–brown colour of the dorsal part of its thorax and abdomen, and the bright yellow scutellum (Figure W1). It has a single pair of transparent wings with a large black dot on the wing tip and black cross-streak on each wing.

Bactrocera tryoni is considered the most damaging fruit fly species in Australia, and is common in the eastern half of Queensland, eastern New South Wales, and the extreme east of Victoria. It is widespread in New Caledonia, French Polynesia and Pitcairn Islands. It was introduced into, but subsequently eradicated from Perth (Western Australia) and Easter Island in the mid-Pacific.

Figure W1. Adult Queensland fruit fly in dorsal (left) and lateral (right) views

Steps 3 and 4. Target and alternative hosts

Bactrocera tryoni is a polyphagous species recorded from more than 113 host plant species in Australia and the Pacific. High risk hosts in the Pacific include breadfruit (*Artocarpus altilis*), guava (*Psidium guajava*), mango (*Mangifera indica*), Tahitian chestnut (*Inocarpus fagifer*), syzygium apples (*Syzygium* spp.) and tropical almond (*Terminalia catappa*). As the survey used a trapping grid, the hosts targeted were those at the grid cross-points.

Step 5. The area

Rarotonga is a volcanic island 32 km in circumference, with a rugged interior of mountains (the highest point is 658 m) covered in native bush, surrounded by a narrow band of agricultural land, which in turn is encircled by a ring of swamps used largely for growing taro. Along the coasts, coconut plantations, beaches, villages and small hotels fringe the island.

The temperature averages 18–28°C in winter and 21–29°C in summer.

Steps 10 and 11. Site choice and sample size

The Ministry of Agriculture of the Cook Islands had in place a Fruit Fly Emergency Response Plan before the incursion, designed to allow a rapid, organised response.

To identify the trapping site locations, a grid of the recommended distances for the traps outlined in the Emergency Response Plan was laid over a map of the quarantined area using a geographic information system. The magnified maps were then used as guidelines to allocate the trapping sites.

Traps were placed in host trees at the sites wherever possible (most of the time), or in non-host trees.

Pre-incursion

There were 15 trapping sites, each with a modified Lynfield trap (Figure W2) baited with *Cue-lure* and methyl ethyl eugenol attractant. All traps were installed at high-risk locations such as the ports of entry, major tourist accommodation sites, diplomatic missions and the rubbish dump.

Figure W2. Lynfield trap

Post-incursion

Cue-lure pheromone traps

Twenty-four hours after the incursion, the Ministry of Agriculture increased the intensity of the trapping network by setting out five additional *Cue-lure* pheromone traps. This resulted in the detection of the second male Queensland fruit fly 280 m from the first site.

The second detection prompted the increase of the trapping radius to 1 km and the whole area was labelled as Zone A. In Zone A, 25 traps were set up in a grid of 300 m^2.

A third Queensland fruit fly picked up in Zone A initiated an increase in the density of traps within the Zone covering an area of 800 m^2 (called the 'intensive zone'). In the intensive zone, 30 traps were set up in a grid of 150 m^2. The Ministry of Agriculture extended the quarantine area to a further 2.5 km radius (Zone B) where 38 *Cue-lure* traps were set in a grid of 500 m^2.

The decisions pertaining to the identification and establishment of Zones A and B were as guided under the Service Outline section of the General and Cook Islands Fruit Fly Emergency Response Plan. The different zoning technically signifies the different response levels, which are initiated depending on the circumstance of the find, and the whole response system is made up of different components that all contribute to trying to contain, control and eradicate the find.

Capi-lure traps

Capi-lure traps were set up at seven selected sites (Figure W3).

Figure W3. Map of the Quarantine Fruit Fly Surveillance Trapping Network

Protein bait spraying

A protein bait spraying program targeting female fruit flies was initiated. This involved spot spraying of trees at about 30 m apart all throughout the infested area covering a distance of about 2.6 km².

Destruction of breeding sites

Fallen fruits, which amounted to approximately 50,000 kg, were also collected from the surrounding area and buried in the quarantined region.

BactroMAT C-L bait stations distribution (male annihilation technique)

BactroMAT C-L bait stations were distributed at 800/km² covering an area of 8 km². The process involved the tying of pheromone baits onto trees at a spacing of 30 m.

Step 12. Timing of survey

The traps were checked every fortnight and the flies caught examined and identified by an entomologist. The lures were re-dipped every 2 months.

Steps 13 and 14. Data and samples collected

Trapping

Any Queensland fruit flies collected in the traps were recorded.

Fruits

A fruit-collecting program was initiated to assess fruit-fly damage and data collected were used in the delimiting survey.

Fallen fruits and fruit picked from trees which could have been infested by the Queensland fruit fly were collected from Zones A and B. Generally, the fruit samples collected were the ones examined and seen to contain symptoms of fruit-fly damage. An average of 25 samples was collected weekly, totalling 940 samples over 14 months.

The fruit were counted, and their weight and site of collection recorded. No Queensland fruit fly were reared from the fruit sampled.

Comments

The success of the Ministry of Agriculture in eradicating *B. tryoni* from Rarotonga rested on the following:

1. There was an existing trapping network that was regularly serviced. This enabled early warning while the fruit fly was still in low numbers.
2. Ministry of Agriculture staff were well-trained in recognising indigenous fruit fly species as well as the high-risk exotic species in the Pacific.
3. Staff responded quickly and in an organised way to the incursion because Cook Islands had an Emergency Response Plan in place. The plan clearly outlined the activities that had to be undertaken upon the detection of an exotic fruit fly species.

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