EDES

SAFE FOOD IN ACP
A PROGRAMME FUNDED BY THE EU

HANDBOOK 12.5

TOPIC 12
Official Controls

5

Surveillance and detection of plant pathogens and pests in the field

COLEACP

The handbooks are tools designed for civil servants in charge of restructuring the food safety system, and for all operators involved in drawing up the food safety policy and organising official controls (qualified civil servants, heads of laboratories, heads of departments in official organisations, those in charge of official controls, trainers, technicians, researchers, experts or company executives). They aim to provide an overview of the main points of a specific subject. All of the topics addressed by EDES during the training sessions are covered in separate handbooks.

The handbooks have been designed and drawn up by the EDES Training Unit in cooperation with the Consortium members.

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fera The Food and Environment Research Agency

France Vétérinaire International

Ministère de l'Agriculture et de l'Agroalimentaire

Natural Resources Institute

anses French agency for food, environmental and occupational health safety

DTU Food National Food Institute

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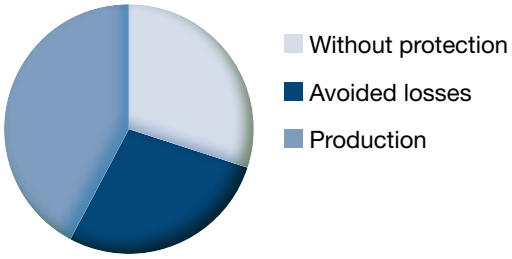
1. Extent of crop enemies and need to protect them

As a result of the combined action of diseases, attacks from pests and competition from weeds, it is estimated that almost **50% of world agricultural production** is lost before or after harvest. Estimated losses, per region and per crop, published in 1965 by H. H. CRAMER were reviewed in 1990 by E. C. OERKE *et al.* for the 8 largest crops (cotton, soya, rice, maize, potato, coffee, wheat and barley).

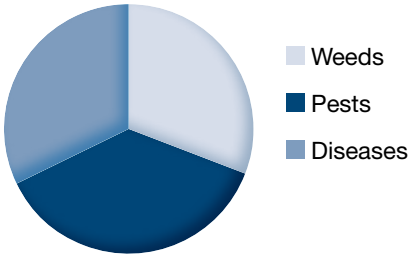
They reveal the **substantial difference** that exists between the "production potential" of the varieties used and the "outputs actually recorded", attributing it mainly to the damage caused to crops by pests, even in regions where the most up-to-date agronomic techniques are used.

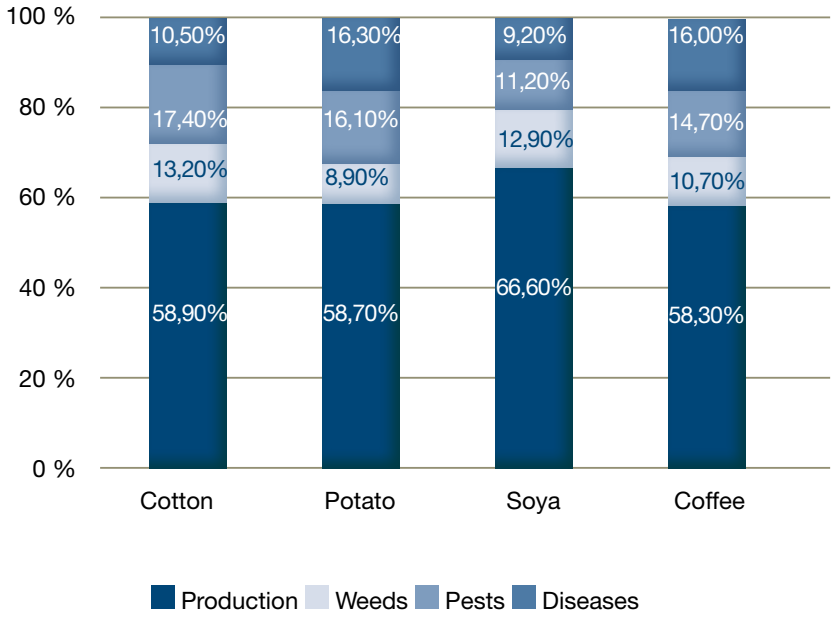
Thus, OERKE estimates that the drop in production is comparable from one region to another when modern production techniques are used, but without any protection strategy. With cotton, for example, output may drop to 15.9% of potential production, compared with 60% currently achieved using various methods of protection.

Relative extent of losses

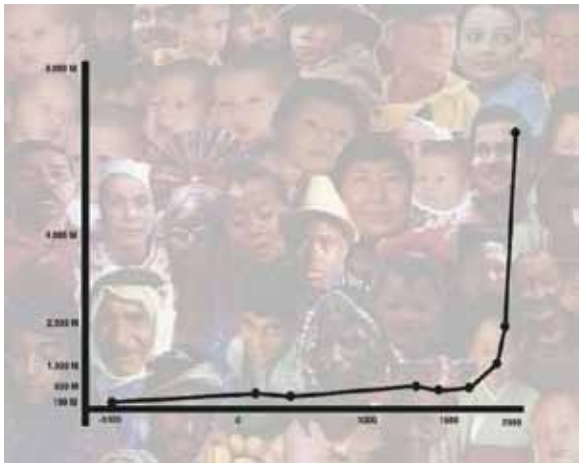


Origin of losses





The explosion of demographic growth over the last few decades, will continue until at least 2100, with the world population rising from over 6 billion to approximately 11.5 billion human beings at the end of the 21st century. What is more, the average increase in the standard of living in some regions where economic growth is strong and rapid also leads to an increase in the world's food needs.



Development of world demography

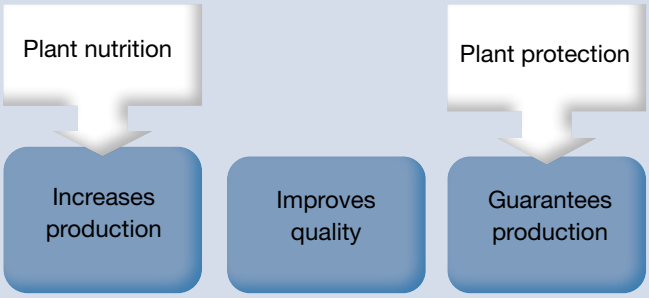
However, there are only two ways of increasing production: **increasing the cultivated surface area, on the one hand**, and **improving productivity per hectare on the other hand**.

Depending on the type of economy in which they operate, and the economic context in which they live, farmers – whose aim is to secure a decent and increasing income for themselves (which is not necessarily the result of maximum productivity per hectare) – exploit one or other of these factors if they can. So, as long as land that is easy to cultivate is available, it may be more advantageous for them to increase the ground they cultivate than to make use of more inputs (fertiliser and pesticides).

Nevertheless, in practically every region of the world, **farmers are now faced first of all with a limitation of arable land available, and secondly with a drop in soil fertility (deterioration of soil, erosion)**.

*Often underexploited, **fertiliser** can deliver immediate production gains as long as varieties selected for their high potential are used.*

***Plant protection** makes use of cultural, genetic, mechanical, biological and chemical methods, used in strategies which are both preventive and curative. The response of crops to the protection techniques is not always as easy to describe as numerous environmental and climatic factors interact.*



This means that the only option they have in the medium and long term is **to increase productivity per hectare** and to **reduce post-harvesting losses**.

In developing countries, food-producing resources, and cereal crops in particular, will have to increase by around 70% by 2020, if the estimated 6.5 billion inhabitants are to be assured dietary security.

Almost all this increased food supply will have to come from countries that are themselves under development. To meet this forecast increase, it will be necessary to see a sustainable increase in the outputs of the main crops of cereals and leguminous plants, and a reduction in farming losses caused by pests and diseases.

Since the possibilities of expanding irrigation and areas suitable for arable farming are limited, **future strategies** ought to be based on **increasing the productivity of the ground and the water resources available**. Undoubtedly, there is no more extensive wastage of these resources than investing time, money and labour in food production only to see these crops swept aside, completely or partially, by infestations with pests, diseases and weeds (see table). Depending on the level of the losses and costs concerned, improving plant health control seems to be an important strategic means of increasing the food resources in existence in developing countries.

Actual production and estimated losses of eight harvests from 1988 to 1990, per parasite and per region (in US $ billion)

Region	Actual production	Causes of losses			
		Pathogens	Insects	Weeds	Total
Africa	13.3	4.1	4.4	4.3	12.8
North America	50.5	7.1	7.5	8.4	22.9
Latin America	30.7	7.1	7.6	7.0	21.7
Asia	162.9	43.8	57.6	43.8	145.2
Europe	42.6	5.8	6.1	4.9	16.8
Former Soviet Union	31.9	8.2	7.0	6.7	22.1
Oceania	3.3	0.8	0.6	0.5	1.9

Source: E. Oerke et al., "Crop production and crop protection: Estimated losses in major food and cash crops" (Amsterdam, Elsevier, 1995).

However, the **incomplete information about the actual losses caused by parasites** and the actual and potential gains of plant health control constitute a great hindrance to formulating a strategy intended to improve plant health control. If all the losses caused by parasites rise to 50%, as indicated by certain researchers, States and organisations such as the World Bank and the CGIAR (*Consultative Group on International Agricultural Research)* must undoubtedly devote more resources to reducing these losses.

Recourse to the inputs available (fertilisers and pesticides) may considerably increase production, and consequently reduce the need to cultivate "marginal" land, protecting the most fragile ground from deforestation, erosion and rapid degradation.

However, when output makes progress thanks to input, selection of variety, irrigation and improvement of crop protocols, the crops also become **more attractive** for the pests and often **more sensitive** to disease or to competition from weeds.

In order to safeguard the production potential, this leads to the **need to use effective methods of monitoring and protecting the crops**.

2. General information about pests, diseases and weeds

A cultivated field or plot constitutes an artificial environment where natural biodiversity has largely disappeared. By concentrating the cultivated species, the farmer encourages populations of pests and epidemics responsible for reducing the output per hectare of the crop. The damage caused to agricultural production and stored foodstuffs by pests, diseases and weeds often represents **over a third of the harvest**.

The agents responsible for these significant losses are mainly **plant-eating insects,** which are easily the most harmful: **nematodes, fungi, viruses and bacteria, not forgetting weeds.** Strategies for protecting crops and methods of controlling these pests are then needed in order to maintain a high level of production.

Pests of the main crops world-wide
(Source: Bayer CropSciences, List of pests, 2001)

Cotton	whitefly, bugs, leaf hoppers (especially dangerous because they are vectors of viral disease), *heteroptera*, *helicoverpa* caterpillars
Maize	wireworm, fruit fly, bug (as vector of viral disease)
Cereals	bugs (especially dangerous because they are vectors of viral disease)
Leguminous crops	bugs, whitefly, leaf hoppers, thrips, caterpillars attacking the leaf and the fruit, leaf miners
Ornamental plants	bugs, whitefly
Rice	leaf hoppers, web moth (*Sparganothis pilleriana*) of rice, aquatic weevil, leaf roller
Stone fruits	bugs, mealy bugs, leaf miners, codling moths, winter moths
Citrus fruits	mealy bugs, bugs, leaf miners, white fly, jumping plant lice
Potato	bugs (especially dangerous as vector of viral diseases), leaf hoppers, Colorado potato beetle
Rape	blossom beetles, stem flea beetles, weevils
Banana plant	Nematodes

Fungal disease affecting the main crops in the world
(Source: Bayer CropSciences, List of pests, 2001)

Cereals	powdery mildew, rust fungus, rhynchosporiosis, septorioses (septoria), and brown spot disease, rot and smut
Rice	pyriculariosis (pyricularia), rhizoctoniae and other diseases of the leaf
Leguminous crops	infected seeds, rust fungus, rotting of fruits and leaves, grey mould, powdery mildew and mildew, diseases of the foliage and the fruits (e.g.: alternariosis, cercosporiosis, etc.)
Potato	mildew, rhizoctonia, silver scurf
Vine	powdery mildew and mildew, grey mould
Pome fruits	scab, mildew, monilia
Stone fruits	monilia
Mango	cercosporiosis
Peanuts	rhizoctonia, sclerotiniosis, cercosporiosis, rust
Banana plant	cercosporiosis (Sigatoka disease affecting the leaf system of the banana plant)
Rape	sclerotiniosis, phoma lingam
Coffee plant	coffee rust

However, a rational and effective fight against crops, pests and diseases **involves minimum knowledge of their lifestyle, their biology and their principal characteristics** in order to be able to identify them both with certainty and as quickly as possible on the basis of the symptoms observed, for an effective and profitable response.

3. Crop infestation, damage in production and at post-harvesting stage

Threats to crop production can arise at an **early stage**, from sowing onwards. **Seeds** that are healthy, high quality and disinfected (not affected by viruses, free from all types of bacteriosis and not colonised by the larvae of insect pests) must be used and **seedbeds** must be maintained under good, healthy conditions, free from nematodes, viruses, insects carrying disease, etc.

Inadequate growing practices (choice of plot and type of soil, inadequate rotation, destruction of beneficial insects, poor weeding and elimination of debris from crops after harvesting, contaminated ploughing tools, harsh pruning, etc.) may also be responsible for massive infestation.

Frequent phytosanitary inspections of plots and orchards, the use of traps, regular soil analyses, clearing weeds from seedlings, observing diseased plants are all necessary in order to detect the start of attacks, to monitor them and if necessary halt their development.

- **Gnawing insects** devour the different parts of the plants (caterpillars of the Lepidoptera family, larvae and adult Coleoptera, grasshoppers and crickets of the Orthoptera family). **Biting-sucking insects** suck up the sap from plants and weaken them. They are also vectors of viruses (whitefly, mealy bugs, zigzag leaf hoppers, greenfly, bugs, thrips).
  Certain insects cause damage to plants because they lay eggs. The development of the larva in the plant tissues is accompanied by consumption of these tissues (fruit flies, leaf-miners that dig tunnels in the leaves). As for underground insects, they attack the roots and tubercles (mole crickets, grey worms). Insects may also be responsible for considerable damage to stored foodstuffs (grains, flour, meat, etc.). Some insects, which are recognised as “quarantine organisms” must be detected in harvested products (ideally prior to their dispatch).

- **Fungi and bacteria** penetrate through the roots, stalks, leaves and fruits through cuts and natural openings, or directly through intact surfaces, resulting in the appearance of marks of different colours or rotting. This damage makes fruit and vegetables unsuitable for consumption and may occur both when they are growing and after harvest.
  Numerous fungi and bacteria are responsible for post-harvesting damage and most viruses infect fruit and vegetables during the growing period and develop during storage, especially under favourable storage temperature conditions. Excluding the direct damage they cause to plants and fruit and vegetables, fungi may also contaminate foodstuffs with the toxins (“mycotoxins”) they release or by inducing in plants products of natural defence (“phytoalexins”). Some of these compounds are particularly dangerous to consumer health even at low concentrations (regulations on acceptable concentrations have been fixed by the European Commission). The invasion of stored products by fungi, thanks to favourable conditions (temperature and/or humidity too high) is generally the cause for contamination by mycotoxins such as aflatoxins or ochratoxins.

- **Nematodes** invade the roots which swell (galls) and the root system becomes nodular; secondary roots develop and the supply of water and nutritional elements no longer takes place: the plant becomes stunted, yellows and withers. In fact, water absorption is very often “impaired”. Assimilation of potassium is reduced, as well as that of sodium, at times. Often a higher concentration of the other mineral elements can be observed in the aerial organs. In the potato, *Ditylenchus destructor* causes a reversal of the relative levels of sucrose and starch.

- As for **weeds**, these may be directly harmful to the crop as they may compete for nutritional elements and water, from the moment the cultivated plant begins to develop. Consequently, this affects the assimilation of chlorophyll in the cultivated plant and therefore its growth. In addition, some weeds grow faster than the crop that has been planted and may therefore be responsible for stifling the developing plant. Finally, weeds may house various parasites (viruses, bacteria, fungi and insect pests) and may therefore be a source of infestation.

Hence the damage caused by the various plant pests and parasites, both when growing and during post-harvest storage, are numerous and vary in importance depending on the state of infestation, the robustness of the plant and the early nature of the intervention which must remain effective and compliant with quality and environmental regulations.

The type of treatment (plant health control) must be appropriate, and must take into consideration the following:

- the organisms to be controlled (efficacy);
- the sensitivity of the crops (selectivity);
- the aim pursued (to limit development, prevent an infestation, eradicate a pest or a disease, etc.);
- regulatory requirements (plant control regulations) and those of specifications (quality standards);
- the skill of the operators;
- safe use and means of protection of personnel;
- targets of competitiveness (profitability of control);
- impact on the environment (durability, protection of bees, etc.).

4. Methods of observing and sampling pest populations in the field

The word “population” is used to refer to all individuals of the same species that occupy a territory (the biotope). The limits of this territory are generally the local geographic region to which this species belongs.

The populations possess a set of characteristics such as the spatial distribution of the individuals, the density, the structure, and so on. The **density of a population** is the number of individuals present per unit of surface area or volume.

Determining population density is important as **the damaging effect of a species in an environment largely depends on its density**… with notable exceptions such as virus vectors!

The methods of evaluating the density of populations, essential for establishing a control strategy, are extremely numerous and may be grouped under 2 main headings: **direct counting** and **indirect methods** (**trapping, extraction**, **etc.**), not forgetting the techniques of diagnosis and sampling.

Choosing a sampling method is a complex process which must be adapted to suit the type of crop being observed. The main stages the observer must carry out are as follows:

1) planning the regularity of recording in line with the pest, the disease or the weeds being targeted and keeping to a schedule of observation and sampling activities;

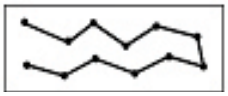
2) determining the units (e.g.: plants, leaves, roots, etc.) and drawing up a plan of the farm’s plots (in order to determine the areas to investigate);

3) determining the pest counting, evaluation and location techniques to be used. Three techniques are mainly used:

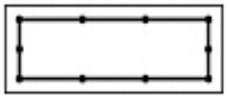
   - counting the pests present according to the different stages of development;
   - observing the damage caused by pests and/or the symptoms caused by diseases;
   - counting the number of seedlings with insects, acarids, nematodes, etc., or that show damage, or symptoms;

4) determining sampling procedures by planning the testing method and by determining the number of samples to be screened:

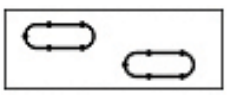
   - if you are trying to detect pests or problems suspected to be **uniformly distributed** or whose distribution pattern is unknown, spread out the sampling points uniformly:



   - if your aim is to detect pests or problems suspected of arising **from external rows**, spread out the sampling points uniformly around the field:



   - if you are looking for pests or problems suspected of being located **in certain portions of the plot**, the sampling points must be concentrated in these sectors:



5) data recording allowing the observer to quantify the populations of pests present and to follow the progress and distribution of parasites during one single season and subsequent growing seasons (particularly with regard to the “threshold of intervention”).

4.1. Direct observation and counting

This method consists of selecting seedlings at random or a particular number of plants along a row of seeds and observing the presence of the pest or the disease on all parts of the plant. This method can be used early in the season and can be applied to the first stages of vegetative development. It has the advantage of not being destructive as no sample of plant material needs to be taken. However, it can only be applied when there is little wind (under 12 km/h). It also requires a good knowledge of the insect system, and the symptoms of the diseases.

In an open environment or one with little plant cover, direct counting can be carried out.
You may need to use a magnifying glass for close observation. In addition, this method can be used for counting birds nests or breeding pairs.

4.2. Trapping and capturing techniques

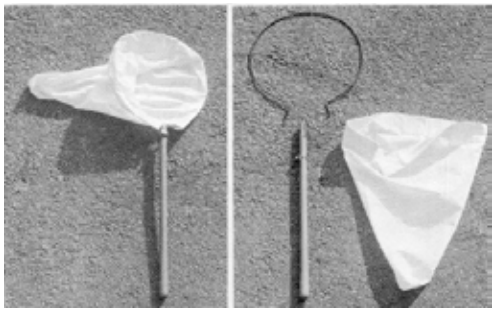
➤ Ground cover

Use of a piece of fabric for soil collection

This method of sampling consists of making the pests fall onto a **piece of light-coloured fabric** measuring approximately 20 cm pegged out **on the ground** at the foot of the plant between two adjacent rows of seeds in order to collect the insects and count them. Obviously, this method is not applicable for insects that fly away rapidly and at the slightest contact (crickets, for example). It is well suited for the Coleoptera, which often let themselves drop when they sense danger and for the caterpillars of Lepidoptera.

Once the pests have been collected, the species can either be identified and counted in the actual field, or be transferred in a suitable container (glass bottle, small plastic tubes…) and then taken to the laboratory to be analysed at a later point. This method is suitable for pests with slow movements but is limited by the size of the seedlings. If they are too small or in senescence, the technique becomes unsuitable. In addition, shaking the seedling can cause leaves to fall outside the perimeter delimited by the piece of fabric and it becomes difficult to count them correctly.

➤ The sweep net



Sweep net

For over a century, this method has been the most widespread for capturing Arthropods harmful to crops. This can be explained by the fact that, in spite of difficulties with standardization, there is no other method capable of capturing so many insects per head and per hour without increasing the cost of the equipment and damaging the crop.

This net consists of three basic elements: the actual **conical net**, the ring which keeps the net open as well as the handle, joined to the ring, made out of aluminium or wood.

Sampling can be carried out **all along a row of plants** by holding the net by the handle and passing it through the foliage. It is also possible to sample the adjacent row as well, by using a zigzag movement. In spite of the fact that this method is very suitable for trapping Arthropods, its results are often variable because of environmental factors such as temperature (which influences the metabolism of insects and therefore their ability to escape), humidity, which has an effect on the microclimate and the location of insects, the position of the sun (the shadow cast by the operator may chase away the insects), the size of the seedlings (which are fragile when small) and the density of the vegetation, which may have a degree of mechanical resistance to the net. When the foliage is wet after rainfall, the net becomes difficult to use.

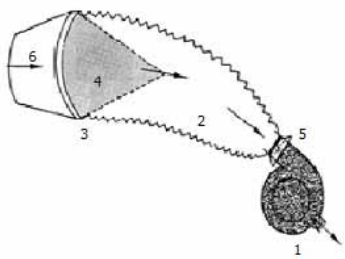
In order to convert the number of insects trapped into absolute estimates of the population, regression methods are used by comparing the population estimates based on insects captured with population densities determined on the basis of an absolute method of sampling such as cage fumigation or collecting the entire plant.

➤ Suction nets

These function by **suction** as fixed or mobile traps for sampling pests within a crop. They have a draft tube (portable fan), a gasoline tank, a flexible air pipe, a collection bag, a small cone and a control.

The sample must be taken in the opposite direction to the direction of the wind. It is possible to take samples over a clearly determined length all along a row by holding the head of the cone horizontally with the rounded part of the net forming an angle of 45° with the row and the top part of the plant.

Diagram of a suction net:



1: fan
2: flexible air tube
3: ring to hold cone
4: net made of tulle
5: filter protecting fan
6: cone adjusting the diameter of the net

Using a suction net

Photo credit: Bill Snyder, Washington State University

This tool is useful for **small-sized pests** capable of being sucked up by the current of air and not frightened by the noise of the apparatus and the movement of the operator. This technique produces good results for flies, some small larvae of Lepidoptera, nymphs and adults of some Hemiptera.

Using this trapping method, the surface of the conical head corresponds to a zone of the field being sampled. The residual population may be determined by direct observation, but better calibration is produced by comparing the results with those of a more absolute method.

➤ Pheromone traps

In market garden crops, fruit and vegetable crops, the caterpillars of butterfly pests and other insects which are parasitic on crops can cause considerable damage. The pheromone trap is a useful tool for detecting insect pests, provides information about the extent of the attack and helps the grower to determine the right time to destroy them.

Pheromones are **chemical signals exchanged between the individuals of the same species** and influence their behaviour. For example, there are sexual pheromones which attract male butterflies located a long distance away from female butterflies. The pheromone trap makes use of this phenomenon to attract insect pests.

The type of trap which gives the best results in practice is the "Delta" trap. This trap consists of a sticky base and a top in hard-wearing, water-resistant material. The trap is hung from a hook placed in the middle of the top. The capsule, containing the pheromones, is located between the top and the sticky base. Males, attracted by the female pheromones, are trapped and remain fixed to the sticky base. By examining this base, the pests can be identified. Counting allows us to obtain an idea of the size of their population and their distribution. Once a certain number of males are trapped, control methods must be started. Pheromones are specific to each insect pest. How long a pheromone's activity lasts depends on its composition, the number of traps used, its concentration and the climate.

Sexual traps are another type of pheromone trap, which use capsules impregnated with a pheromone similar to the pheromone of the female of the pest sought. There are sexual traps for lepidoptera, but also for other pests, such as certain diptera.

There are two main categories of trap: **traps for detection** and **traps for extensive trapping**.

- *Traps for detection* (or "monitoring") are used to **indicate when a pest is beginning to fly**. Hence the user is able to use a sustainable approach when applying chemical or biological treatments (for the introduction of trichogram wasps for example). They are mainly sticky traps, so the males attracted by the synthetic pheromone become trapped. This type of trap may be used for numerous pests in tree crops, field crops, market garden and ornamental crops, viticulture, and so on.
- *Traps for extensive trapping*, consisting of a funnel and a receptacle which holds the butterflies, are used to **capture large quantities** of lepidoptera. Using a synthetic attractant, the aim is to capture and destroy a large number of insect pests on crops. This method is specific and environmentally-friendly. This method of control via extensive trapping is able to control pest populations in the medium term, but is not effective against all species. It is of particular use against Lepidoptera pests.

➤ Sticky traps

- *Delta traps*

The delta trap, generally made from recyclable plastic or cardboard, is impregnated with sexual pheromones but also with glue which traps the insects. This trap has a small entrance to prevent insects from escaping.

- *Wing traps*

The wing trap is also made of paper resistant to bad weather, with sticky internal surfaces, wide openings for increased diffusion of the pheromone in the surrounding environment and bait with pheromones inoffensive to other insects. However, the efficiency of these traps is poor.

- *Cone traps*

This trap uses synthetic pheromones fixed to the base of a cone net as bait. This cone is placed at ground level in high grass and the insects which are trapped in it accumulate in a reservoir on top. These traps are the most effective on the market, although the trapping period is 4 to 8 days longer than that for light traps. They are almost 4 times as effective if placed in the middle of the vegetation and not above it. They are also effective outside the plot, during the pest's first cycle (lepidoptera).

➤ Water pan traps

A capsule containing pheromones is fixed to a string above a container holding a "wetting agent". This liquid, consisting of soapy water, reduces the water repelling nature of the cuticle of insects, which can no longer remain on the surface and hence sink more easily to the bottom of the container. The liquid in the container must be changed regularly (every week) for an optimum yield. They are as effective as light traps, but unfortunately they are very dependent on atmospheric conditions, either evaporating in dry conditions or becoming diluted in rainy conditions.

➤ Other traps

- **Black light traps** are especially effective for Lepidoptera and other nocturnal insects. The light produced by a 15 W bulb attracts butterflies or other insects which fly into the metal plates impregnated with soap. The insects then slide into a container full of soapy water and remain trapped there. These traps are among the most effective where there are high densities of insects. However, they do not contain pheromones, so are not very selective. They actually attract not only the female lepidoptera of a given species but also other species, or even other insects. These traps can therefore make counting the insects difficult if similar species are mixed together.

- The most effective trap is still **the coloured bowl** (**yellow**) **full of soapy water** (**water traps**) which collect the insects attracted by the colour. Water has an attractive effect in the sense that the insects move towards places where humidity indicates the presence of water. The reflections from solar and atmospheric light on its surface also have an effect of attraction and finally this hides the walls of the dish to an extent and the insects focus on the water. The insects are attracted over a distance of 30 to 40 cm. These traps have numerous advantages such as their simplicity and low cost, the ease of collecting the insects (the contents of the dish are poured into a funnel with a removable plastic tube at the end. The contents of this are then collected in a container and alcohol is squirted in). This keeps the insects in good condition (apart from butterflies). Finally, they do not require any source of energy. The specific nature of the captures should be noted, as the insects are usually attracted by specific wavelengths.

- The **Malaise trap** resembles a canvas tent in which flying insects "are lost"; passively directed to the higher end of the "roof" before being collected in a container fixed to this end.

- The **emergence trap** is able to collect populations of ground Arthropods. It can be used in a *dry extraction* form or a *wet extraction* form:
  - **Dry extractors** (Berlèse apparatus; Tullgren apparatus; Tullgren apparatus combined with repellents such as naphthalene) use a source of heat and are suitable for micro and macro-arthropods.
  - **Wet extractors** (Barmann, Seinhorst or Milne apparatus) often consist of a sieve containing the sample of earth onto which *water* is poured, with the entire mixture then being heated by a lamp placed over it. The oxygen content drops and the animals fall down a tube to escape from the heat, reaching a container of cold water where they are collected. These wet extractors are suitable for samples of nematodes.

There are also mechanical methods of **extraction by directly examining samples of earth** with or without colorant (nematodes), by means of the **direct examination of sections of soil**, by means of extraction by **dry sifting** (coleoptera), by means of extraction by **floating** (nematodes, acarids, molluscs), by **wet sifting and flotation** (Ladell, Aguilar, Bernard and Bessard methods, Salt and Hollick method), by means of **centrifuging and flotation**, by **sedimentation**, by **elutriation**, and by **maceration of the substrate**.

4.3. Absolute sampling methods

Accurate methods of estimating population densities are needed to produce management programmes for pest populations. The methods described above depend on environmental and human conditions and other biological factors. The validity of the data collected using these techniques can only be judged on the basis of their efficacy when these are compared to a more reliable and less costly sampling method. The two methods described below are based on isolating a population over a known surface area.

The first method is **cage fumigation** (cage made from wood, plastic or lightweight metal). The cage must also have a very small opening at the top in order to allow the application of the fumigant as well as a collection plate at the bottom. An aerosol pack containing 20% of a pyrethrinoid makes an excellent fumigant. 5 to 8 seconds of spraying are often sufficient to have a “knock down” effect on Arthropods inside the cage. Without removing the cage, the operator inserts an arm through the injection cylinder and energetically shakes the plant. The cage is then removed and the insects are collected at the base.

Sampling by fumigation (A: Choice of plant, B: Sample)

The second method consists of **collecting the whole plant** using a sampling cage measuring 1.8 x 1.8 x 1.8 metres, made of net and mounted on a cubic support. An opening which can be closed is made on one of the sides of the cage. This allows access to the inside of the cage. The cage is placed over the sampling location by two operators one of whom goes into the cage with an aspirator, labels and plastic bags. The aspirator is used to suck out the insects from plants, which are then pulled out and placed in bags provided for this purpose. The plant debris (leaves, branches) are also collected and placed in separate bags. The cage is left in place for 1 to 2 hours in order to collect the individuals that have fallen into holes or have been enveloped in dust when moving towards the edges of the cage. The methods of dry extraction allow the insects to be “removed” from the plant debris and the soil.

Using a method of statistical regression, in the form of $y = \beta x + \alpha$ is used, with y corresponding to the number obtained using the sampling method employed and x the number obtained using the absolute sampling method (fumigation or collection of plants), the efficacy of the sampling method selected is tested.

Illustration of the sampling method for the whole plant (sampling cage):

1: Transporting the cage to the field
2: Putting the cage in place
3: Collecting the insects that have fallen to the ground after sampling and bagging up the plants
4: Removing the cage and sampling surface

5. Methods of observing fungi and bacteria

5.1. Methods of observing symptoms

Accurate observation of symptoms and their development in time and space constitutes the first stage of the diagnosis. The symptoms are sometimes sufficiently defined and specific to allow the cause of a disease to be correctly identified without requiring other analyses: this is the case with certain traditional afflictions such as rust, mildew and smut.

However, more often than not, the situations encountered are complex: different agents may induce similar symptoms while, on the other hand, the same agent may produce symptoms which vary according to the situation. In addition, the most visible symptoms do not necessarily appear at the primary site of infection; for example, certain pathogenic agents responsible for necrosis of the radicular system or of vascular tissues (**primary symptoms** or **causal symptoms**) cause secondary withering or shrivelling of the aerial parts (**secondary symptoms** or **consequential symptoms**).

The period when symptoms appear, as well as the **climatic circumstances** which preceded their appearance, is extremely important when diagnosing a disease caused by a fungus or bacteria.

The **previous cultivation** as well as the different **operations carried out within the crop** may interfere with the initiation and development of symptoms; mineral fertilisers (doses and dates of application), plant health treatments (doses, commercial names, equipment and spreading techniques), work on the soil, the date of sowing or planting and the origin of batches of seeds or organs of propagation will be taken into particular consideration.

The history of the field may reveal circumstances which favour the appearance of symptoms, even after several years. Likewise, demarcation between symptoms may correspond, after several years, to the boundaries of plots with a different history. Spatial distribution may provide elements which are useful in the diagnosis: valley bottoms and sides of hills with a Northern exposure are locations which are particularly favourable to damage by fungi developing in rather more humid and cold conditions. Dips are often areas where symptoms of root asphyxia are seen.

The way in which diseased plants are distributed in the crop is also able to shed light on the way in which the causes of the infection are transmitted or on their transmission. Distribution in lines parallel to the seeds reflects human origin (compaction of the soil associated with the passage of machines, overdoses of manure or plant health products, linear distribution of an inoculum by tools). Diseased plants in an area at the entrance of a field may correspond to deposits from bags of manure (scabies caused by *Streptomyces scabies* in areas where calcium-containing fertiliser is stored); diseased plants distributed in small groups forming spots distributed at random in the field may reveal that the virus has been transmitted by aphids. On the other hand, a disease that appears year after year, in the same place and whose affected surface area is mainly increasing in the direction in which the soil is worked, suggests a microbial origin or transmission of the virus by nematodes or by fungi.

> At this stage in the diagnosis, it is important to **pick up on every clue** that will make it possible to determine the biotic or abiotic nature of the problem, by taking samples. When the cause of the disease cannot be established on-site, **samples need to be taken for subsequent analyses**.

This sampling must be carried out with the greatest of care, as its quality will determine the success of the later stages (observations under the microscope, isolation, etc.). It is always preferable to **sample entire plants** (including roots), rather than limiting the sample to the parts which seem damaged in order to identify the causal symptoms. It is also a good idea to take samples at various stages of progress of the disease, particularly in plants showing early symptoms (with a view to isolating the pathogenic agent and of observing its fruiting bodies) or showing an advanced stage of the infection (presence of the parasite's survival structure).

5.2. Methods of diagnosis in the laboratory

Various laboratory methods are used to make the diagnosis. They are the reserve of specialists and of well-equipped and, if possible, certified laboratories.

The laboratory techniques can be split into **three categories** depending on their aim:

- detecting infectious parts of the pathogenic agent (**biological methods**);
- revealing immunogenic molecules synthesised by the pathogenic agent (**immunological methods**);
- detecting sequences of nucleic acids that are specific to the genome of the pathogenic agent (**molecular methods**).

➤ Biological methods

A simple close examination of the surface of the samples of diseased plants using a **binocular magnifying glass**, or of a sample under the **microscope**, is sometimes sufficient to reveal carpophores of fungi or bacterial exudates whose presence may be grounds for diagnosing a parasitic disease.

Practical example: the "exudates method" to confirm the presence of *Ralstonia solanacearum* (brown rot).

This method is used for certifying potato seedlings.
Ralstonia solanacearum is a soil bacterium, a Gram-negative plant pathogen, responsible for brown rot. Present on every continent, particularly in tropical and subtropical regions, the bacterium is stored in the soil where it can survive for several years. It penetrates through the roots and propagates through the vascular system; it is spread by irrigation water (surface water) or by the seedlings. It colonises the xylem, causing bacterial rot or vascular bacteriosis in numerous host plants from the Solanaceae family (tomato, nightshade, pepper, aubergine, tobacco, etc.) and other plants as well.

Method of detection (extract from: *Draft of the plan to control the certification of potato seedlings, CDE - Lux Development – AIDCO, 2009*):

Pull up the plant and check whether:
- The main stem and/or the roots are being attacked by an insect.
- The stems are rotting around the neck (Erwinia).
- The main stem is giving off an exudate:
  - Equipment: transparent glass + knife + bottle of clear water
  - Method: cut off the main stem 5 cm above the neck and soak it in a glass of water. Wait 1 to 3 minutes to check for the presence of white filaments coming out of the vascular tissue.

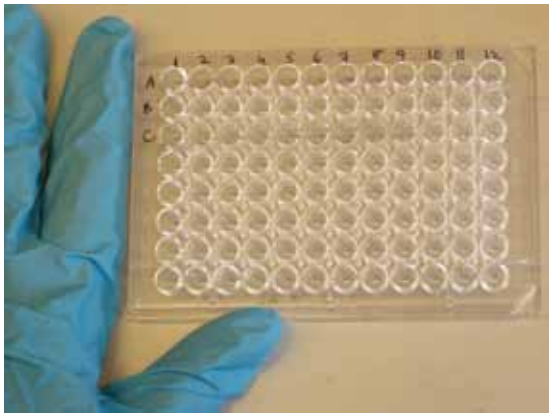
Result:
If filaments are observed, the plant is definitely suffering from *Ralstonia solanacearum*, which means that the soil, tubercules and nearby plants must be removed.

In more complex cases, a procedure will be used to **isolate the agent**; the different stages of this work involve: (1) choosing a plant sample; (2) disinfecting its surface, depositing it in a nutritional environment and (3) observing the growth of the uncontaminated culture.

The final identification can extend as far as inoculation of the agent which has been isolated. This method only applies to the pathogens capable of multiplying on the medium in vitro (fungi, bacteria).

Biological methods of diagnosing **obligate parasites** (viruses, phytoplasma, etc.) are based on a series of operations: descriptions of the symptoms observed, **transmission of the infectious agent to host plants** and symptoms, determination of the range of host plants and the symptoms they express, observation under the microscope (possibly electronic), extraction and purification (in the case of viruses and viroids).

➤ Immunological or serological methods



Numerous molecules of a pathogenic agent may behave like antigens by causing, in the lymphatic tissues of warm-blooded animals, the formation of antibodies with which they react specifically. Several serological techniques make use of this property; they use both polyclonal antibodies, and monoclonal antibodies.

Enzymatic marking of these antibodies has allowed the development of protocols capable of detecting phytopathogenic agents and quantifying them (**ELISA test**).

The ELISA (acronym for *Enzyme Linked ImmunoSorbent Assay*) test is an immunological test intended to detect and/or assay a protein in a biological liquid.

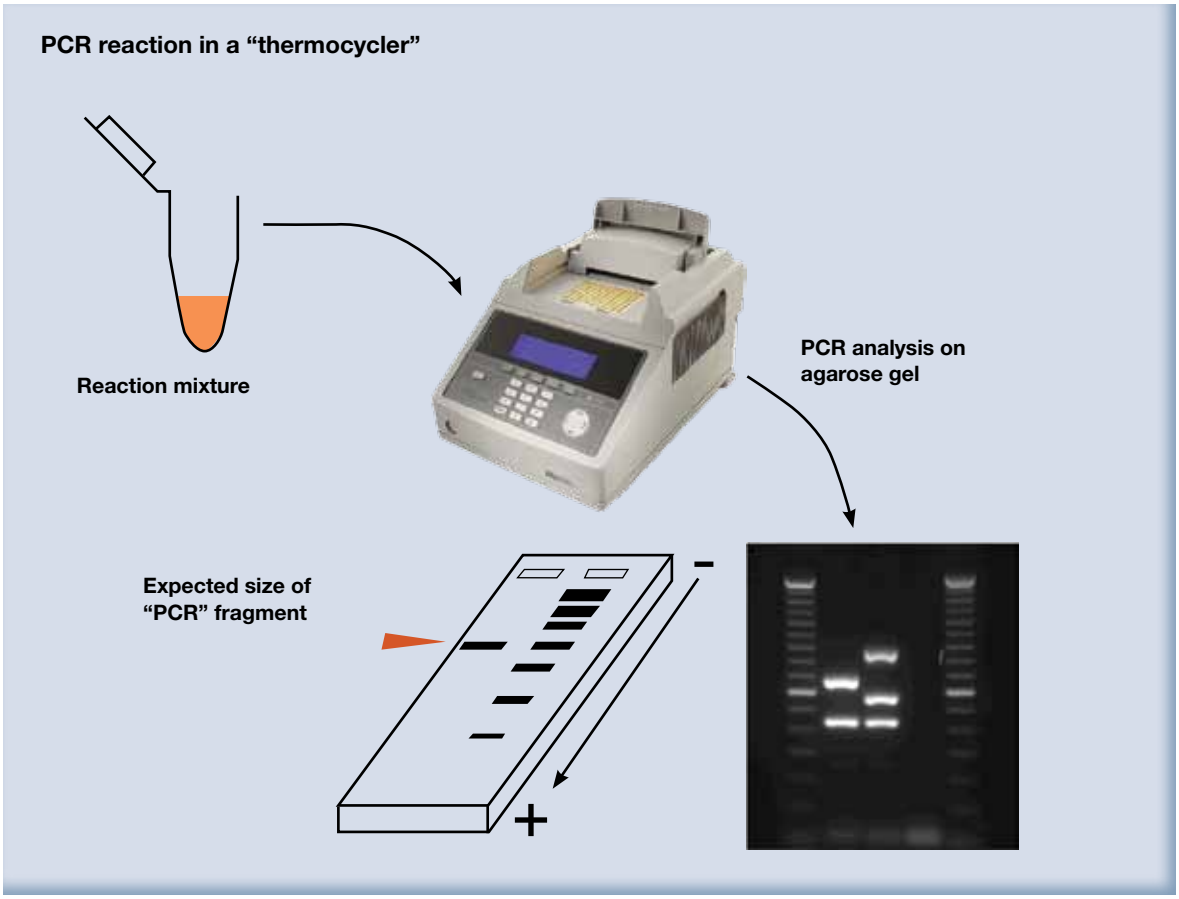
The main advantages of immuno-enzymatic tests are their **sensitivity** and their **ease of use**. However, it may be difficult to obtain antibodies in the case of diseases with an ill-defined etiology, or disorders whose agent cannot be cultivated in vitro or purified easily.

➤ Molecular methods

Serological methods cannot be used to diagnose diseases caused by viroids. In this case, diagnostic techniques are used based on **the analysis of sequences of nucleic acids** from infected plants using electrophoresis in polyacrylamide gel or on the characterisation of nucleic acids by molecular hybridation.

Recourse to **molecular amplification**, via chain polymerisation (**PCR** - *Polymerase Chain Reaction*), has made it possible to push back **the boundaries of sensitivity** of diagnostic techniques based on the detection of specific sequences of nucleic acids. The aim of the technique is to make a large number of copies of a given segment of DNA (e.g.: amplifying a specific region of a nucleic acid of the virus to be detected, in order to make the virus “visible”). In order to make this possible, a series of reactions allowing the replication of a matrix of double-stranded DNA is repeated in a loop. In the course of the PCR (*polymer chain reaction*), the products obtained at the end of each cycle act as a matrix for the next cycle, so the amplification is exponential.

This amplification produces a **band on a gel** (see figure) that is specific, on account of its size, to the virus we are trying to reveal. If this technique is properly developed, it is both very sensitive (amplification possible as soon as there are a few cells infected with the virus alone) and very specific. The PCR reaction is extremely rapid and only lasts a few hours (2 to 3 hours for a PCR involving 30 cycles).



Reading results on the polyacrylamide gel

6. Detection of quarantine organisms (sampling) and plant health certificates

Protecting crops against their enemies is a **question of general interest**, which requires an **organisation** capable of **preventing the introduction** of a plant pathogen into a given country or area and of issuing the certificates required to market plant products.

In the first case, **the crops in unaffected countries or regions are the priority** of the regulations. In the second case, the main aim of the regulations is to **protect the product being marketed and its user**. The merchandise may not constitute a risk to plant health as such, but it may be a carrier of harmful organisms.

Since March 2005, new European regulations and new obligations imposed on **wood packaging** have come into force[1]. The Directive aims to bring European legislation in line with the provisions of the "International Regulation for phytosanitary measures - ISPM N° 15" of the FAO relating to the "Directives on the Regulation of Wood Packaging Material in International Trade". From now on, any wood packaging material originating in a third country used in the export of foodstuffs to Europe must be the subject of plant health certification. The targeted products are mainly wood packaging material in the form of bins, boxes, crates, as well as pallets, bin-pallets and other loading stations. The third-party countries which carry out the export are therefore obliged to carry out a plant health examination of the wood products they use and to provide proof that the wood has been stripped, has undergone an appropriate thermal treatment at 56 °C, or appropriate fumigation, or even chemical impregnation under pressure.

6.1. International Plant Protection Convention (IPPC)

The International Plant Protection Convention (IPPC) was signed in 1951 under the aegis of the FAO. The convention was reviewed in 1997 in the wake of the Agreements of the Uruguay Cycle of the World Trade Organisation (WTO), particularly the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement). The convention is the result of international collaboration on plant protection and the prevention of the dissemination of agents harmful to plants (animals, viruses, prokaryotes, fungi, weeds). It reaffirms the need for plant health measures which are technically justified, transparent and compliant with the SPS Agreement and it supplies a framework which guarantees that the plant health regulations put in place have a scientific basis justifying their application and that they do not constitute a hidden restriction on international trade.

One of the most important measures as far as the IPPC is concerned consists of drawing up the inventory of harmful organisms which are particularly dangerous, whose introduction to the Community must be prohibited, and harmful organisms whose introduction through certain plants or plant products must also be prohibited.

6.2. Risk evaluation procedures

Any plant health regulation must be based on a **risk evaluation** in accordance with a procedure which the FAO has codified (**PRA procedure**). The "Pest Risk Analysis" is a process consisting of **evaluating biological evidence** or other scientific or economic data to determine whether a harmful organism should be regulated, and the severity of any plant health measures to be taken against it.

This procedure concerns harmful agents which meet the definition of a quarantine organism. According to the IPPC definition, a quarantine organism is 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.

1 Directive 2004/102/EC of 5 October 2004, amending Annexes II, III, IV and V of Council Directive 2000/29/EC on "*protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community*".

The risk evaluation procedure will take into consideration criteria of a geographical, biological and economic nature into consideration such as the probability of establishment of the pathogen, its potential spread and the economic consequences of its introduction in a geographical region in which it is absent. Once the level of risk has been evaluated, all the means likely to reduce this risk to an acceptable level are envisaged. The principle of the "minimum impact" recommended by the IPPC is to adopt quarantine measures whose restricting nature is proportional to the level of risk.

One of the essential requirements of a risk evaluation is to be able to have available accurate and reliable information about the geographical distribution of the agent under consideration. In this respect, the FAO, the RPPO (Regional Plant Protection Organisations) and various international organisations (CAB, EU, etc.) publish documents that make it possible to monitor the emergence of pathogenic agents and their distribution worldwide.

6.3. Monitoring quarantine organisms

Inspecting consignments is an essential element of managing plant health risks, and it is the plant health procedure most frequently used to establish whether or not harmful organisms are present and/or their compliance with the plant health requirements of the destination market.

➤ Basis for sampling

Each batch must be checked. A whole dispatch cannot always be inspected, which is why the phytosanitary inspection generally involves samples from the dispatched lots.

A "**dispatch**" may comprise one or several batches of products. When it involves more than one batch, the inspection aimed at establishing compliance will possibly give rise to several different visual examinations, which involves **sampling the batches separately**. In this case, the samples relating to each batch must be isolated and identified so that the batch concerned can be clearly identified if a subsequent inspection or analysis shows that it does not comply with plant health requirements.

Sampling within a batch begins with **identification of the most suitable sampling unit** (e.g.: *n* fruits, unit of weight, bag, carton) depending on the product[2]. As a rule, fruits or vegetables are inspected during sorting in the station or during packaging.

When compiling the sampling plan, the level of acceptance for a quarantine organism must be fixed at **zero**, and the **calculation of the number of samples** must be carried out on this basis.

In order to calculate the number of samples to be examined (**n**) out of a population (= one batch) of fruit/vegetables (**N**), 2 possibilities must be taken into consideration:

- Sampling of **small batches**: the size of the sample (n) > 5% of the size of the batch (N).
  In this case, when a unit from the batch is sampled, **the probability** that the next unit sampled will be infested changes. Sampling, without any replacement in a small batch, is based on a hypergeometric distribution.
- Sampling of **large batches**: the size of the sample (n) < 5% of the size of the batch (N).
  In this case, for large size batches which have been adequately mixed together, sampling is based on a binomial distribution or a Poisson distribution.

Please note!

Even if no individual (egg, larva or adult) is detected in the sample examined, the probability that an organism is present, even at a very low level, remains. The threshold of monitoring in principle is not in itself a guarantee of plant health compliance.

2 Distribution not approved

Simple random sampling is used. In practice, the **operator uses Tables from Standard ISPM 31** – *Methodologies for sampling of consignments* (FAO, IPPC 2008).

➤ Calculating the number of units to examine in small batches

In **Standard ISPM 31** – *Methodologies for sampling consignments* (FAO, IPPC 2008), the operator will find 4 tables[3] indicating the minimum number of samples to be examined according to the number of fruits/vegetables in a batch and the confidence level selected (80%, 90%, 95% or 99%). As a general rule, a confidence level of 95% is deemed to be sufficient.

The size of the sample is determined from the level of detection and the degree of efficacy.

Minimum sizes of the sample for a 95 per cent confidence level, according to the size of the batch, with the level of acceptance being 0:

Number of units in the batch	P = 95% (confidence level) % level of detection x efficacy of detection				
	5	2	1	0,5	0,1
25	24*	-	-	-	-
50	39*	48	-	-	-
100	45	78	95	-	-
200	51	105	155	190	-
300	54	117	189	285*	-
400	55	124	211	311	-
500	56	129	225	388*	-
600	56	132	235	379	-
700	57	134	243	442*	-
800	57	136	249	421	-
900	57	137	254	474*	-
1 000	57	138	258	450	950
2 000	58	143	277	517	1553
3 000	58	145	284	542	1895
4 000	58	146	288	556	2108
5 000	59	147	290	564	2253
6 000	59	147	291	569	2358
7 000	59	147	292	573	2437
8 000	59	147	293	576	2498
9 000	59	148	294	579	2548
10 000	59	148	294	581	2588
20 000	59	148	296	589	2781
30 000	59	148	297	592	2850
40 000	59	149	297	594	2885
50 000	59	149	298	595	2907
60 000	59	149	298	595	2921
70 000	59	149	298	596	2932
80 000	59	149	298	596	2939
90 000	59	149	298	596	2945
100 000	59	149	298	596	2950
200 000 and over	59	149	298	597	2972

3 The tables (ISPM 31) are available on the IPPC site:
http://www.ippc.int/file_uploaded/1323947615_ISPM_31_2008_En_2011-11-29_Refor.pdf

Example of application:

For a batch of approximately 2,000 fruits, if we estimate that on average the percentage of infested fruits is 2%, 143 fruits must be sampled (approximately 7% of the fruits).
The confidence level of 95% means that on average only 5% of infested fruits will not be detected.

➤ Calculating the number of units to examine in large batches

In **Standard ISPM 31** – *Methodologies for sampling of consignments* (FAO, IPPC 2008), the operator will find 2 tables (one according to the binomial law, the other according to the Poisson law) indicating the minimum number of samples (n) to be examined in the large batches depending on the confidence level chosen (95% or 99%). The size of the sample is determined from the level of detection and the % of efficacy.

Minimum sizes of the sample for 95 or 99 per cent levels of confidence, according to the values of efficacy, with the level of acceptance being 0:

n according to the binomial law					
% of efficacy	P = 95% per cent (confidence level) % level of detection				
	5	2	1	0,5	0,1
100	59	149	299	598	2 995
99	60	150	302	604	3 025
95	62	157	314	630	3 152
90	66	165	332	665	3 328
85	69	175	351	704	3 523
80	74	186	373	748	3 744
75	79	199	398	798	3 993
50	119	299	598	1 197	5 990
25	239	598	1 197	2 396	11 982
10	598	1 497	2 995	5 990	29 956

n according to the binomial law					
% of efficacy	P = 99% per cent (level of confidence) % level of detection				
	5	2	1	0,5	0,1
100	90	228	459	919	4 603
99	91	231	463	929	4 650
95	95	241	483	968	4 846
90	101	254	510	1 022	5 115
85	107	269	540	1 082	5 416
80	113	286	574	1 149	5 755
75	121	305	612	1 226	6 138
50	182	459	919	1 840	9 209
25	367	919	1 840	3 682	18 419
10	919	2 301	4 603	9 209	46 050

Minimum sizes of the sample for 95 or 99 per cent levels of confidence, according to the values of efficacy, with the level of acceptance being 0:

n according to the Poisson law					
% of efficacy	P = 95% per cent (confidence level) % level of detection				
	5	2	1	0,5	0,1
100	60	150	300	600	2 996
99	61	152	303	606	3 026
95	64	158	316	631	3 154
90	67	167	333	666	3 329
85	71	177	353	705	3 525
80	75	188	375	749	3 745
75	80	200	400	799	3 995
50	120	300	600	1 199	5 992
25	240	600	1 199	2 397	11 983
10	600	1 498	2 996	5 992	29 958

n according to the Poisson law					
% of efficacy	P = 99% per cent (confidence level) % level of detection				
	5	2	1	0,5	0,1
100	**93**	231	461	922	4 606
99	94	233	466	931	4 652
95	97	243	485	970	4 848
90	103	256	512	1 024	5 117
85	109	271	542	1 084	5 418
80	116	288	576	1 152	5 757
75	123	308	615	1 229	6 141
50	185	461	922	1 843	9 211
25	369	922	1 843	3 685	18 421
10	922	2 303	4 606	9 211	46 052

Example of application:

For a batch of approximately 400,000 fruits, if we want to be able to detect with 95% confidence an infestation of 1% of fruits with an efficacy of 80%, 353 to 375 fruits must be sampled, i.e. approximately 0.1% fruits to be examined. The 95% confidence level means that on average only 5% of infested fruits will not be detected.

6.4. Plant health measures implemented

➤ Quarantine and eradication measures

Plant health regulations may prohibit importation, submit their authorisation to a prior plant health inspection or make disinfecting of the merchandise obligatory. Once the first source of a quarantine agent has been declared, we can try to prevent its spread by means of a regulation imposing the detection of the disease, the application of certain measures with a view to eradicating or limiting it, or sometimes even abandoning growing some sensitive species or varieties.

➤ Certification

The plant health certificates are **issued by a qualified authority** which must guarantee that the product is free from any disease covered by quarantine laws. Issuing plant health certificates is therefore entrusted to technically qualified operators duly authorised by the national organisation for the protection of plants to act on its behalf and under its control, possessing the necessary knowledge and information so that the importing authorities can accept the plant health certificates of other States as reliable documents. For Europe, the Plant Health Certificate must be compiled according to the Model shown in Annex VII of **Directive 2000/29/EC**.

For the so-called “quality” organisms, certification guarantees the user a product suitable for the use for which it was purchased.

➤ Cost/benefit of regulatory measures

A preventive plant health regulation will only be adopted after having compared the cost of applying these administrative measures and whichever of the means of control which must be implemented if the disease has been introduced to the country.

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