TOPIC 5C

QUARANTINE, ERADICATION AND LEGISLATION

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THE PRODUCTION, RELEASE AND PERFORMANCE OF HEALTHY PLANTING MATERIAL OF VEGETATIVELY PROPAGATED CROPS

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ABSTRACT

Several types of pathogens can be perpetuated and disseminated through the vegetative propagation of their hosts. Pathogen-free material can be obtained by selection, heat treatment or tissue culture, in some cases with the aid of chemotherapy. Multiplication of pathogen-free material must be done under careful control. Methods of quick multiplication by cuttings or by in vitro propagation have been developed in recent years. The advantages of using pathogen-free material and various propagation methods are discussed.

INTRODUCTION

Vegetative propagation of plants has the great advantage of reproducing highly heterozygous varieties true-to-type, but it has the disadvantage that pathogens tend to accumulate in the propagated material, decreasing yield potential and reducing the longevity of the plants, if proper precautions are not taken.

The pathogens whose dissemination occurs mainly or partly through vegetative propagation of their hosts are mostly viruses and viroids, but spiroplasma, mycoplasma-like and rickettsia-like microorganisms, bacteria and even fungi may also be disseminated in this way. For several viruses vegetative propagation is the most important means of spread. For instance, grapevine leafroll virus spreads little in vineyards and no vector is known, but the disease is prevalent in vineyards throughout the world.

The increasing concern for the health of vegetatively propagated plant material originated from the study of potato viruses, whose elimination from elite stocks led to important increases in productivity. Since then, more and more pathogens and even animal pests have been found that are spread by vegetative propagation in many crops and ornamentals. Avoiding these unwanted organisms depends on two conditions: obtaining pest- and pathogen-free material of good cultural quality and multiplying it free of contamination until it can be planted in the field. Fulfilling these two conditions requires a third one: a reliable and sensitive method for detecting and identifying the disease.

It should be emphasized here again, as it has been done already by several authors, that no method can guarantee that a plant or a clone is entirely virus-free, or free of all pathogens. This would necessitate knowing all viruses or other pathogens that can infect the plant and the availability of detection methods that are not limited in their sensitivity. Neither condition can be realized with certainty.

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OBTAINING HEALTHY PLANTING MATERIAL

Several methods can be used to obtain healthy material for vegetative propagation, depending on the type of pathogen and host:

- Visual selection, indexing and/or serological tests
- Heat therapy
- Meristem- or shoot-tip culture *in vitro*, or other tissue culture method
- Heat treatment and meristem-tip culture
- Cold treatment and meristem-tip culture
- Chemotherapy and meristem-tip culture

Choice of source plants in the field by visual selection, followed by indexing and/or serological tests

This method can be used only when some plants have escaped infection. With perennial plants such as grapevine or fruit trees, better chances of success are often encountered in old plantings, or in countries where vectors are absent or not very active. With some viruses that are not entirely systemic, green cuttings or buds taken from infected plants and rooted or budded onto healthy rootstocks may give healthy plants.

Heat therapy

There are basically two types of heat therapy: brief treatments at relatively high and almost lethal temperatures for a short time, from a few minutes to a few hours, and longer treatments lasting several weeks or months, or even over a year, at temperatures ranging from 35 to 40° C.

Brief treatments at high temperature are done in warm water or aerated steam. This type of heat therapy was used first in 1921 with sugarcane affected by sereh disease, a suspected virus disease, and it is still applied mostly to this crop plant. Setts are treated for 2-8 hours at 52-54° C before planting. Several diseases due to bacteria and mycoplasma-like microorganisms can be eliminated in this way. Brief heat treatment has been also used for curing gladiolus bulbs from aster yellows and potato tubers from phyllody (mycoplasma-like microorganisms), and also to eliminate the fungus *Phytophthora cinnamomi* from grapevines. In a review on heat therapy of virus diseases of perennial plants, Nyland & Goheen (1969) list 29 viruses or virus complexes that can be eliminated by brief heat treatment. Most of them are now known to be mycoplasma-like organisms or spiroplasms. There is so far no clear evidence that any true virus has ever been inactivated *in vivo* by brief hot water or hot air treatment. Several attempts with different viruses gave negative results in recent years.

The first virus to be eliminated by prolonged warm air treatment was potato leafroll virus (Kassanis 1949). In a series of experiments started in 1950 at East Malling Research Station, Posnette (1953) showed that three strawberry viruses could be inactivated when potted plants were treated for 8 days at 37° C in a warm air chamber. These findings opened the way to an important new field in applied virology. The methods and results of heat therapy have been reviewed by Hollings (1965) and by Nyland & Goheen (1969).

Plant material can be heat treated as potted plants, as buds of the variety to be cured budded onto a healthy rootstock, as small plantlets grown aseptically in vitro, or as tubers, bulbs or other propagules. When the virus or viruses cannot be eliminated from the whole plant, shoot tips are detached after a sufficient period of treatment and rooted either under mist or aseptically in vitro, or grafted onto a healthy rootstock. Many different heat chambers have been described. The essential points are good controls of temperature and humidity and suitable illumination. Heat treatment temperatures range from 35 to 38°C, depending on plant species and type of virus, exceptionally up to 40°C for citrus. Fluctuating temperatures have been found advantageous in some cases. The length of treatment necessary varies according to the virus.

Long-term warm air treatment has been very useful in eliminating many virus diseases of economic importance, several yellows diseases due to mycoplasma or spiroplasma, and diseases attributed to viruses, but whose agent has not yet been isolated and identified. Nowadays, however, heat treatment alone is used less than 20 years ago, and tends to be replaced by meristem-tip culture, either alone or combined with heat therapy, as a way of obtaining pathogen-free material.

Tissue culture

Plant tissue culture has facilitated great improvement in the production of pathogen-free planting material of vegetatively propagated plants. The first important step was the discovery that meristem-tips excised from virus-infected plants and cultured in vitro could give virus-free plantlets (Morel & Martin 1952, Hollings 1965, Quak 1977), even with viruses that could not be eliminated by heat therapy. The second step was the development of methods for a rapid in vitro multiplication of plantlets originating from meristem-tips or from shoot apices by proliferation of axillary or adventitious buds and their rooting in a suitable growth medium. In the last 10-15 years, these techniques have expanded very quickly in all countries, at the research level and in commerce.

Meristem-tip culture

The most widely used tissue culture method for obtaining pathogen-free plant material is meristem-tip culture. This term, as suggested by Hollings (1965) usually refers to the meristematic dome plus the first pair of leaf primordia, i.e. a length of 0.1 - 0.5 mm. The meristem alone seldom grows into a plantlet. When explants larger than meristem-tips are used, the percentage growing successfully may increase, but the chance of obtaining plantlets free of viruses or other pathogens is usually less. However, several authors have obtained virus-free plants from apical explants (which can be referred to as shoot tips) of up to several millimeters. Meristem-tips are usually taken from terminal or axillary buds, but entire plants have been regenerated from root meristems. Methods and media used for meristem-tip culture or for other types of tissue culture have been described in the excellent reviews of Hollings (1965) and Quak (1977) and in the books of Reinert & Bajaj (1977), Ingram & Helgeson (1980) and Conger (1981), to quote only a few of them.

Whether they are eventually multiplied by conventional methods or by in vitro cloning, plantlets regenerated from meristem-tip culture are

usually propagated initially in vitro to build up a clone. This can then be tested for freedom from viruses or other pathogens after the plantlets have been adapted to normal soil culture conditions.

Recovery of meristem- or shoot-tips by grafting onto a healthy rootstock in vitro

Meristem- or shoot-tips that cannot be rooted easily in vitro can be grafted, directly after excision or after a certain period of growth, onto a suitable rootstock grown aseptically. This method has been used successfully with citrus, peach and grapevine after heat treatment of the mother plant.

Other possibilities of obtaining healthy material by tissue culture

Pathogens, especially viruses, are not usually present in all cells of plant tissue. As whole plants can now be regenerated from single cells, there is a chance that some of the plantlets derived by tissue culture from isolated protoplasts, callus cells or other cells from infected plants may be healthy. However, there is a risk of genetic variation in plants derived in this way. Healthy plants can be also obtained from apomictic seedlings of polyembryonic cultivars of citrus, or from nucellar tissues of monoembryonic cultivars. Nucellar plants, however, have the disadvantage of exhibiting undesirable juvenile characters. Meristem-tip culture remains therefore the most widely used tissue culture method for obtaining pathogen-free clones of cultivated plants.

Heat treatment and meristem-tip or shoot-tip culture

Heat treatment of donor-plants prior to excision and culture of meristem- or shoot-tips has been found advantageous in many cases, as larger explants can be used with greater chances of successful growth and virus elimination. Meristem-tips or shoot-tips can also be exposed to heat therapy in culture.

Cold treatment and meristem-tip culture

With plants such as clover, which is very sensitive to high temperature, keeping the donor-plant at 10°C for 2-4 months prior to explant excision, instead of heat treatment, has been used with some success (Barnett et al. 1975). This method seems useful for eliminating potato spindle tuber viroid from potato (Lizarraga et al. 1980).

Chemotherapy and meristem-tip culture

Although no antiviral substance has yet given clear success in eliminating viruses from whole plants, virazole (ribavirin) added to the growth medium of tissue culture was helpful in several cases (Van Aartrijk & Blom Baarnholm 1982, Cassels & Long 1982). Schuster (1982) mentions a synergistic antiviral effect of virazole with 2,4-dioxo-hexahydro-1,3,5-triazine (DHT), which might improve the efficiency of virazole in tissue culture. Amantadine at 50-100 mg/l in the culture medium of chrysanthemum meristem-tips made it possible to eliminate chrysanthemum stunt viroid in c. 10 % of the plantlets, whereas no viroid-free plantlets were recovered from control cultures (Horst & Cohen 1980). Antibiotics have been used to control systemic infection by bacteria in in vitro cultures of philodendron (De Prest et al. 1980).

TESTING THE MATERIAL FOR PATHOGEN FREEDOM

Whatever method has been used to obtain healthy material, it is necessary to ensure that the target pathogens have been eliminated and tests must be repeated during clonal multiplication. For viruses, plant indexing and/or serology are the usual methods. The recently developed methods of ELISA (Clark & Adams 1977, Clark 1981) and immunosorbent electron microscopy (Milne & Luisoni 1975) are particularly sensitive. The great advantage of ELISA, besides its high sensitivity, lies in the possibility of automating much of the process, in the rapidity of the test and its good reproducibility. The use of monoclonal antibodies (Gugerli & Fries 1981) greatly improves the method. ELISA can be also used with spiroplasms, bacteria and even fungi (Clark 1981). Viroids can be detected and identified by indexing, by polyacrylamide gel electrophoresis or by nucleic acid hybridization, which is the quickest and the most sensitive method (Owens & Diener 1981). Methods for detecting and identifying bacteria have been assessed by Schaad (1980).

Unfortunately, several virus-like pathogens and the mycoplasma-like microorganisms have not yet been isolated and purified, and serology can seldom be used to detect them. Indexing, which takes weeks, months, or even years for some fruit tree or grapevine "viruses" is thus the only detection method available.

MAINTENANCE, MULTIPLICATION AND DISTRIBUTION OF HEALTHY MATERIAL

Healthy material can be maintained in screenhouses or glasshouses well protected from outside contamination and with suitable soil disinfection. Isolation from sources of infection can be provided outdoors by distance, altitude or specific environment (island, desert or mountain area). Tissue culture also maintains healthy material free of contamination. Meristem-tips or other types of tissue cultures can be stored for long periods at about 4-6°C without growth and resume growth when returned to higher temperature. It is also possible to deep-freeze the cells or tissues in liquid nitrogen (- 196°C), using suitable cryoprotectants.

When multiplying healthy material, care must be taken to avoid contamination. Roguing infected plants, timely burning of haulms in the case of potatoes, spraying with insecticides and soil disinfection before planting are some of the commonly used ways of protecting plants in the open. Isolation from sources of contamination as mentioned above is essential. In order to avoid contamination by the bacterium Erwinia carotovora var. atroseptica, potato plants can be propagated as cuttings in the glasshouse. With woody plants, especially fruit trees and grapevine, the classical methods of propagation are often slow and difficult. Rooting green cuttings under mist can hasten the process.

In the last 10-15 years, the use of tissue culture for propagating agricultural and ornamental plants has revolutionized vegetative propagation, as reflected in the numerous papers, reviews and books published on this subject (see Reinert & Bajaj 1977, Sharp et al. 1977, Conger 1981). In most cases, proviously tested clones are multiplied by enhancing the proliferation of axillary or adventitious buds which are severed from the initial explant, rooted in a suitable medium and later transferred to soil. Plantlets can be also produced from single cells by somatic embryogenesis.

The advantages of vegetative propagation by tissue culture are numerous: rapidity of multiplication, economy of space, absence of any risk of external contamination if proper precautions are taken, easier transfer of clones through quarantine, possibility of storing the plantlets for several months at low temperature. There are also a few disadvantages: need of skilled personnel, more costy equipment than for traditional methods, need for access to a scientific institute or the availability of a good scientific staff in the establishment, risk of genetic variation of the material. Any mistake in the choice of the initial plant material, whether it is a genetic defect or a latent infection, is quickly amplified to proportions that can be disastrous if it is not detected early. The decision to shift from traditional methods to in vitro propagation depends on these different factors, but economic criteria predominate.

Distribution of tested material is usually made through a certification scheme. Before any clonal material is distributed on a large scale, it should be tested for its agronomic performances in the conditions where it will be cultivated.

RESULTS OF USING HEALTHY PLANTING MATERIAL

The elimination of pathogens, especially viruses, from vegetatively propagated planting material has achieved considerable increases in the yield of many crops. This improvement is particularly valuable with potato, strawberry, raspberry and other small fruits, fruit trees, grapevine, rhubarb, hop, and with many ornamentals such as orchids, carnations, gladiolus, dahlias, lilies, etc. It will certainly have an impact in future on several tropical crops in which viruses are still a limiting factor. Besides the increase in yield potential, elimination of pathogens often improves quality, as with grapevine leafroll disease that lowers the sugar content of grapes and the quality of the wine produced.

Another important advantage of healthy planting material is its better uniformity in growth and its greater longevity. With fruit trees and grapevine, using virus-free rootstocks and scionwood gives a better percentage of bud- or graft-take and a higher proportion of saleable plants of good quality, to the great benefit of nurserymen and orchardists.

For geneticists and plant breeders, the use of healthy material is advantageous for two reasons. Transfer of plant germplasm between countries is facilitated, especially if it is done with material in vitro. With perennial plants such as grapevine where clonal selection within traditional varieties is important, elimination of viruses and virus-like pathogens removes an important variable, leaving only genetic differences between clones.

There are, however, a few problems linked with improvements in the health of vegetatively propagated material. As the cost of obtaining and testing healthy clones is rather high, their number tends to be low, resulting in a narrowing of the genetic diversity available.

With several crop plants, quality and quantity of yield are negatively correlated. This effect is particularly important with grapevine, and

virologists are often criticized for providing growers with clones having excess vigour or that are too productive, at the expense of quality, after viruses have been eliminated. It can be argued, however, that quality control must be done by growers and not by viruses.

The increased vigour of healthy material can induce a greater sensitivity to attack by fungus diseases. Again with an example from grapevine, elimination of viruses may result in more compact grapes that are more susceptible to grey mould caused by the fungus Botrytis cinerea.

Abnormalities linked with propagation in vitro have been mentioned occasionally. Multi-apexing of strawberries (Anderson et al.1982) seems to by influenced by the proportion of growth substances in the medium. Malformations of the flowers of freesias have also been mentioned (Oertel 1979). Heat treatment of grapevine by the in vitro method of Galzy (1964) resulted in important changes in the varietal characteristics of several grape cultivars (Mur et al. 1972) influencing leaf shape, pilosity, colour and fertility. These changes seem to result from the prolonged culture of the plantlets in vitro rather than from heat therapy (Grenan 1982). Such observations emphasize the necessity of a careful check of the quality of the plant material during the multiplication.

Despite these few minor problems, the use of healthy, pathogen-tested material of vegetatively propagated crops has been of great benefit and will be especially valuable in countries where food is lacking. In countries where agricultural productivity is already high, improved planting material with even better performance can be introduced at little additional cost and with benefit to the consumer as well as to the farmer.

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SEED HEALTH, SEED TESTING AND SEED CERTIFICATION

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ABSTRACT

Seed provide a ready means by which plant pathogens are introduced into new agricultural zones, survive between growing seasons, are selected as specific strains and are disseminated in plant populations. To evaluate the health status of seed lots, it is necessary to know the economic importance of seed-borne pathogens and the conditions under which they develop. Seed health testing provides the only means of detecting and identifying seed-borne pathogens. It is essential to set up control procedures and to check if systems for producing healthy seed have been effective. Seed certification provides the best way to ensure high quality seed, with the particular guarantee that traded seed lots have been subjected to health control with respect to the most harmful seed-borne pathogens.

INTRODUCTION

Knowledge of the health status of seed lots exchanged in national and international trade is a fundamental feature in evaluating their quality. Seeds constitute in fact the easiest way by which plant pathogens are introduced into new agricultural zones, survive between growing seasons, are selected as specific strains and are propagated from foci of infection in plant populations. Moreover, seed-borne pathogens cause types of damage which are not always recognised by users, such as seed death, seedling and plant abnormalities or decreased seed vigour.

Such effects, which are produced as early as the first stages of seedling development, are particularly detrimental to crop establishment and harvest. Relationships between seeds and pathogens are complex. It is frequently difficult to detect the presence of one infected seed among several thousands, yet such contamination can introduce a disease into a new area or start an epidemic which may have very harmful economic effects.

SEED HEALTH

Seeds carrying pathogens may not show any visible symptoms and it is often necessary to use laboratory tests or to examine seedlings during germination to prove the presence of a pathogen. Consequently, insufficient research has been carried out in the field of seed pathology, particularly on the evaluation of economic losses originating from seed, the setting up of simple, quick, accurate and cheap seed health testing methods, and the application of protection and production measures ensuring the health of seed lots. This is particularly alarming because most crops are grown from seed and it is important to know the risks encountered on transferring pathogens from one place to another, by their perpetuation between seasons and by their transmission to further generations during the multiplication process (Baker 1980).

Seed-borne pathogens are of various kinds. Best known are the bacteria, fungi and viruses, but seed lots can convey nematodes, insects and parasitic plants, and also weed seeds. Until now, insufficient importance has been given to the fact that such weed seeds can be the source of disease

epidemics in crops (Anselme 1981).

The severity of a seed lot infection is the consequence of the virulence of the pathogen, the concentration of inoculum, its location in or on the seed (superficial or internal, near or far from embryo) and the speed of its dissemination. Thus seed health status can only be correctly evaluated by a thorough knowledge of the biology of pathogens and of the methods used for their detection and identification.

In practice a methodical approach is needed in drafting seed health regulations and their application.

Quality of seed lots

The presence of a pathogen in a seed lot affects quality mainly due to the associated risk of potential loss. This risk can be expressed at emergence (dead seeds, abnormal seedlings, damping-off), by formation of foci in the field and by later spread of epidemics. It is also possible that the pathogen remains latent and only develops later when plant development or environmental conditions are favourable.

Economic importance

The economic impact of a pathogen is evaluated according to the losses produced, in numbers of units (men, animals or plants) whose development is affected or which are destroyed directly or indirectly by parasitic attack. It is not usually until users have had repeated losses that the economic importance of pathogens is established (Anselme 1981). In fact users often make, from their own experience but sometimes uncritically, a cause-effect connection between presence of a seed-borne pathogen and crop losses. However, the evaluation of the health status of seed lots may vary according to the user including seed multipliers, the seed trade, farmers, certification agencies. When a country wishes to set up protective measures against specified pathogens it is necessary to perform studies and surveys to determine, according to experience and by means of the information and methods of protection available, those against which action must be taken as a matter of priority. In practice it is not possible to apply protective measures to all the potentially harmful organisms, so it is necessary to make a choice in relation to an agreed risk.

An adversary so designated will be better detected and fought. Once an inventory has been compiled and choices have been made, it becomes easier to define regulations to be applied within the framework of national (certification) or international (quarantine) seed exchange (Anselme & Mathys 1978).

Development of pathogens

The incidence of a pathogen depends on certain conditions including presence of the pathogen, susceptibility of the host plant and a favourable environment for development. It is the reason why information for evaluating the economic importance of a pathogen must include data on its potential for multiplication in relation to climatic conditions both in the zone where the seeds were produced (multiplication of inoculum) and in the zone where the seeds will be used (development of epidemics). Such knowledge allows evaluation of the risk from seed lots infected in various proportions (Anselme 1981).

SEED TESTING

Once harmful pathogens have been listed, it is important to define for each of them the methods to be used for their detection and identification (Neergaard 1977).

Such a method must create the most favourable conditions for detecting and then identifying pathogens in seeds.

Some pathogenic organisms can be detected by visual examination of seeds. This is the easiest case, provided that a close correlation has been demonstrated between the pathogen and the symptoms observed, as with bean anthracnose. However, while presence of symptoms can be characteristic of a pathogen, their absence does not necessarily mean that the seed lot is healthy.

Pathogens (bacteria, fungi, viruses, seeds of parasitic plants like broomrapes) can be present and invisible to the naked eye. It is then necessary to adopt other techniques which may be as complex as they are varied. Incubation on nutritive media facilitates the appearance of characteristic fructifications of fungi (conidia, pycnidia, mycelium and seedling symptoms), while washing seeds allows the identification of pathogens present on the seed surface (spores). Methods consisting of spraying healthy seedlings with suspensions obtained from presumably infected seeds soaked in water can be used to demonstrate the presence of bacterial pathogens (de Tempe & Binnerts 1979). Immunofluorescence methods are quicker and more accurate (Schaad 1982) but necessitate the use of refined technology and complex materials (Van Vuurde & Van Henten 1982).

Viruses can be detected by indirect methods using indicator plants (Rohloff & Marrou 1981), or more recently by serological methods including ELISA and others linked to electron microscopy (ISEM) (Lange et al. 1982).

Setting up of methods

Methods used for seed health testing should ensure that different laboratories obtain similar results within statistically accepted limits of tolerance, for random sampling from a homogeneous seed lot. Rules defining sampling methodology and the setting up of seed health testing methods are described respectively in the International Rules for Seed Testing and in the terms of reference of the Plant Disease Committee of the International Seed Testing Association (ISTA 1976).

In order that a seed health testing method can be recommended in the ISTA rules, it must comply with the two tests of repeatability and reproducibility :

a) Repeatability - once a method has been set up, several samples from the same seed lots are tested either by several analysts in the same laboratory or by one analyst, ignoring sample identity. Results obtained must be within the statistically accepted tolerance limits (Anselme 1982).

b) Reproducibility - the same test must be performed successfully by different laboratories likely to perform the same seed health tests.

Obtainable information

The competence of a seed health testing laboratory must be clearly known to users, a laboratory being specialized only for the detection and identification of a limited number of designated pathogens for which it has

good experience (bacteria, fungi, viruses). Requests for seed health tests must only concern the detection of pathogens on a restricted list established by the profession, or within the framework of certification or quarantine regulations.

The detection of several pathogens can generally be performed only by the use of different methods. Thus the detection of net blotch, loose smut and barley stripe mosaic in barley requires the use of three separate methods (Anselme 1983).

Expression of results

According to the pathogens selected and methods used, results are expressed as percentage of infected seed or as presence or absence of a pathogen in the seed sample (Champion & Mecheneau 1979). In some cases, it is necessary to be able to transform a qualitative result into a quantitative one (Maury et al. 1982). In both cases accuracy depends on the method used and on the number of seeds tested. Information given as whole percentage units is generally sufficient for fungal pathogens. In the case of bacteria and viruses, an accuracy of one per thousand or more is necessary. Several thousands of seeds must then be tested together or sequentially. Quantitative information allows better evaluation of the risk of disseminating a pathogen, but qualitative information may be sufficient to show whether or not the seed lot should be multiplied.

SEED CERTIFICATION

The only way to avoid introducing a pathogen into a crop through seeds is to use uninfected seed lots. If this is impossible to ensure, the risk should be evaluated.

Selection of pathogens

The three means of control used to avoid introduction and spread of a pathogen in a crop from seeds are :

1) resistant cultivars,
2) healthy seeds,
3) chemical treatments.

Whatever the method used, it is necessary to select the pathogen to be controlled because it may be impossible either to obtain resistance to several different organisms, or to eliminate several pathogens by the use of only one chemical. Seed crop production based on accurate knowledge of the pathogens to eliminate and of the health status of the nucleus used for multiplication is required to obtain healthy seed lots. Health testing of the seed produced gives the possibility of checking the result obtained and may show which chemical to use.

The choice of pathogens against which protective measures must be taken constitutes the most difficult decision for breeders, seed firms and certification agencies which take the responsibility of distributing high quality seed lots.

Production of healthy seeds

In general, pathogens must be detected in the earliest stages of breeding and seed multiplication (Hewett 1978), when control is most easily achieved. Since some sowing methods have become very sophisticated, it is necessary to pay particular attention to the health of the original material:

technically - to ensure the success of the new methods used (precision drilling, pelleted seeds) and economically - to protect the financial investment represented by the marketing of a high quality seed lot. While large quantities of seeds of industrial crops are exchanged annually between continents, a considerable number of small seed lots are also exchanged privately or by breeding centers, experiment stations or production organizations, without any tests. This mass dissemination worldwide constitutes the ideal means of spreading seed-borne diseases. Healthy seed lot production thus depends on the systematic implementation of progressive multiplication of small quantities of seeds, whose health status has been carefully checked before and after each multiplication, performed in a controlled climatic environment.

Tolerance

Due to climatic variation, it may be impossible to produce pathogen-free seed stocks annually for a given species. At the most, districts of production can be defined where climatic conditions are not favourable for the spread of pathogens. Thus a systematic comparison between flax seed produced in France consistently showed in 8 years (1957-1964) an average of more seeds infected by *Botrytis cinerea* in the oceanic zone (11 % infected seeds) than in the continental one (0.9 %), on an average of 300 seed samples tested annually. Moreover, observations between 1964 and 1966 indicated that the development of the same pathogen on sunflower seeds was linked to the evapotranspiration-rain value (Anselme & Champion 1970), from which a 5 % infection tolerance has been established in the French regulations for seed certification (Règlement technique 1979).

It should be understood that standards are established for average climatic conditions and that they cannot apply to unusual circumstances (Hewett 1981). Control methods and particularly chemical seed treatments must be considered in setting up tolerances (Jørgensen 1979). Thus a minimum infection rate can be accepted with or without treatment of the seed lot, the health test being used to establish the critical levels around which seed lots are accepted or not. Thus the Austrian regulations indicate an index of 10/35 for *Septoria nodorum* on wheat ; this means that with less than 10 % infection, seed lots need not be treated, that it must be done between 10 and 35, and that lots with more than 35 % are rejected (Bundesgesetzblatt 1973).

Information for users

Users pay attention to seedling emergence in their own fields and generally attribute difficulties to the poor health status of the seed. Such an attitude may be unjustified, especially if there is no information on the health status of the seed lot before its use. Confusion is possible between losses due to a pathogen which is usually seed-borne but which could have reached the seedlings in other ways (soil or aerial transport) and losses directly attributable to the use of an infected seed lot.

Attention paid to the quality of sowing and emergence can result in pathogens concealing each other. Some pathogens like viruses may be very insidious and cannot be detected, particularly when a seed lot has been imported. This is the reason why national regulations for certification and quarantine are closely related.

In addition, it is necessary for extension services to explain how to evaluate information on the health status of a seed lot. Such indications, linked to knowledge of other aspects of seed quality and to the conditions of its use allow an evaluation of the risk encountered and a decision on how to employ the seeds with maximum guarantees (unrestricted use of the lot,

chemical treatment, supervision at emergence, application of treatment, or roguing during growth).

CONCLUSION

Production of healthy seed stocks is not simply an end in itself, and the establishment of phytosanitary standards and the implementation of certification schemes are justified economically.

It is mainly by long-term action that effective results will be obtained, even if some user do not appear to appreciate the advantages. This entails increased costs in producing healthy seed lots, the need to evaluate potential risk and the interpretation of information to decide on the treatment or use of an infected seed lot. Rules for certification, together with production methods for healthy seed lots, are then indispensable. One must however remain vigilant concerning the further spread of seed-borne organisms. To be well understood and efficient, regulations must be dynamic and practical ; this means that every action must be based on accurate scientific knowledge in order to be able to give the vital information and instructions for the correct use of protective measures and to check that effective results have been obtained.

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5C—S3

SAFEGUARDING THE INTERNATIONAL EXCHANGE OF PLANT GERMPLASM

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ABSTRACT

Plant pests and pathogens of economic and/or quarantine significance can be moved far over natural and man-made pathways. These pathways are reviewed here with emphasis on the transfer of plant germplasm between regions. This constitutes a hazard particularly for organisms that need living plant material to complete a life cycle or which have no efficient means of natural spread. The man-made pathways are discussed in relation to origin and destination. The concept of pest risk analysis and safeguarding are discussed with particular application to the export and import of plant germplasm by Agricultural Research Centers supported by the Consultative Group of International Agricultural Research. The criteria used and assessments of these centers are discussed and recommendations presented to facilitate the rapid but safe exchange of germplasm.

INTRODUCTION

Plant pests[1] of economic and/or quarantine significance can be moved far by natural means and also in, on, or along with articles moved by man. Of these articles, living plants or plant parts present the greatest hazard with: 1) obligate parasites that require living plant material to complete a life cycle, 2) facultative parasites that require living plants at least during part of the life cycle, or 3) other pests with no natural means of long distance spread.

When all the ways of moving plants and plant parts are considered, the importation of plant germplasm has the very greatest potential for facilitating transfer of exotic pests. Much of the germplasm is collected in the wild, in markets, or in remote agricultural areas where the pests have not been well characterized. These collection sites may be in the center of origin or diversity for the host and its pests.

The risk is not significantly ameliorated if the collector is a scientist. Even if unhealthy-looking plants are avoided latent or obscure pests are likely to be collected, particularly in vegetative propagules. Much of the plant material is collected leafless as seeds, fleshy underground storage organs, or dormant scions or plants. Consequently, neither observation by a collector nor inspection by a plant quarantine officer using facilities and equipment available at a port of entry would detect any latent or obscure pests present.

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[1] The term "pests" is used herein to refer to all harmful organisms or agents that infect or infest plants.

The response of many countries to the risk is to prohibit the introduction of hosts from areas where 'high risk' pests are known to occur. The extent to which 125 countries prohibit certain genera from at least one country has been reviewed (Kahn 1982). The 38 most frequently prohibited crops or genera were listed as were the 10 most frequently named fungi, viruses, nematodes, bacteria and insects. In addition, 11 methods of prohibition or exclusion are described. However, despite these prohibitions many countries allow scientific material to enter, albeit under special safeguards.

Principles, methodology and suggested approaches to facilitate the international transfer of genetic resources have been reviewed already (Kahn 1977). This review discusses the risks associated with natural and man-made pathways, especially those involving germplasm. A concept of pest risk analysis and the role of a system of independent safeguards to decrease the chance of transfering pests are also discussed. The policy of implementing most of the safeguards at origin rather than on entry is proposed. The application of these concepts is discussed in relation to exchange of plant germplasm by the International Agricultural Research Centers (IARC) supported by the Consultative Group of International Agricultural Research (CGIAR).

PATHWAYS

Many, but not all, pests of economic and/or quarantine significance can move far by natural means. Others may move over a sequence of short distances for a long period until reaching a natural barrier. However, many viruses and bacteria without mobile vectors, most nematodes, many fungi without airborne spores and some insects such as scales are seldom spread far naturally, although they are readily moved along man-made pathways. The various groups of pests and the natural and man-made pathways over which they may move are listed:—

Pest Groups

+ insects
  mites
  slugs
  snails
+ fungi
+ bacteria
+ nematodes
+ parasitic plants
+ noxious weeds
  protozoa
  algae
+ viroids
+ viruses
  ricksettsia
  spiroplasmas
  mycoplasma-like organisms
  Vertebrates
  e.g. rodents, birds

Natural Pathways

* winds and storms
* air currents
* ocean currents
  spashing rain
* surface drainage
* seed dispersal
  root grafting between plants
  fliers (insects)
  self-locomotion (spores, cells, etc.)
  vectors (such as insects, mites, fungi, nematodes, parasitic plants)
* other carriers such as animals including birds

Man-Made Pathways

containers
* mail
* baggage
  dunnage
* "smuggling"
# used vehicles
# common carriers
  growing media

* imported forest litter

* agricultural cargo
# non-agricultural cargo
# packing materials
# soil, sand, gravel
* plants and plant parts
  microrganism cultures
  nursery practices
  manufacturing based on
    agricultural products

+ = denotes groups which contain some members which are seed-borne.
* = denotes direct pathways for seeds
# = denotes pathways for seeds as contaminants

These pathways can be considered in relation to origin and destination. At the source, whether a pest enters a natural pathway depends on biological variables including life cycle, environment, population density, etc. Whether a pest enters a man-made pathway depends on such factors and also on regulatory considerations. These include the scale and speed of transit, control procedures and other safeguards.

PEST RISK

The concept of pest risk analysis (Kahn 1979) depicts diagramatically the interaction of biological, economic , and political factors in quarantine situations (Fig. 1). Pest risk is rated as low to high along the abscissa and entry status from liberal to conservative along the ordinate. Pest risk refers to the actual or perceived threat of moving pests of quarantine significance along man-made pathways. The actual or perceived risk is based on biological variables as reviewed previously (Kahn 1977, 1979). Entry status is the summation of regulations, policies, procedures, or decisions by quarantine officers that govern the movement of material along these pathways.

A biologically sound position is reached when pest risk is matched with attitudes towards entry status. Thus, sound positions are high pest risk and conservative entry status or low pest risk and liberal entry status. Non-biological positions are low pest risk and conservative entry status (sometimes presented as an economic position) or high pest risk and liberal entry status (sometimes the policy of importing governments during periods of famime, economic stress, or food shortage).

SAFEGUARDS

If there are effective safeguards along man-made pathways, pest risk can be lowered and entry status can become more liberal. As the number of effective safeguards is increased, particularly if they operate independently, the risk is decreased further. An example of a safeguard is inspection on entry. An example of two independent safeguards is inspection on entry with treatment as necessary. An application of this concept in the importation of citrus fruit for consumption has been reviewed (Kahn 1979).

For most commercial and many scientific importations of plants or propagules, safeguards are stipulated by most importing countries. These include: 1) A permit authorizing importation from a specific geographic area 2) a phytosanitary certificate issued by the quarantine service of the exporting country, 3) inspection upon entry and 4) depending on pest finds, a treatment if available.

When the importing country regards these safeguards as inadequate, entry is prohibited; or, if a pest is found during inspection at entry, the consignment is destroyed or prohibited. Some high risk plants are prohibited by several coutries even for scientific purposes, e.g., Cocos nucifera, coconut, which is subject to diseases of unknown etiology occurring in certain geographic areas. However, most of the genera that are prohibited may, nevertheless, be admitted under special permits provided additional safeguards are taken. Sometimes the permit will require an added declaration on the phytosanitary certificate that a prescribed safeguard such as virus indexing or inspection during the growing season has been implemented at origin. In other instances, additional safeguards may be required after entry such as growing imported plants in isolation or out of season in a glasshouse.

Quarantine officers who have a conservative attitude to importations of some of the higher risk genera as germplasm might be more liberal if more safeguards were adopted. If a country has its own quarantine station it has more leeway by providing its own safeguards after entry. However, if not, the importing country has the choice of prohibition or seeking to increase the number of safeguards at the source.

This paper considers the matter of increasing the number of safeguards at origin and uses the importing and exporting of germplasm by International Agricultural Research Centers (IARC) as an example.

SAFEGUARDS AT THE INTERNATIONAL AGRICULTURAL RESEARCH CENTERS

After serving as a consultant to FAO and as a representative of APHIS, the author reviewed the policies, procedures, and safeguards by which the IARC's working in cooperation with the quarantine services of their respective host countries export and import plant germplasm. The review included 19 crops at six of the seven IARC's in the CGIAR network.

The following criteria were used in the review for germplasm imports in evaluating how the IARC and the quarantine service protected the agriculture of the host country from the entry of exotic pests of quarantine significance: 1) Regulations of the quarantine service of the IARC host country; 2) Requirement for a permit from the quarantine service of the IARC host country; 3) Requirement by the quarantine service of the IARC host country for a phytosanitary certificate from the quarantine service of the exporting country; 4) Flow of germplasm through a 'filter' of the quarantine service of IARC host country; 5) Inspection of germplasm upon entry; 6) Seed health testing if warranted; 7) Treatment upon entry as available; 8) Safeguarding features at the site where seeds or propagules are first grown after entry; 9) Growing season inspection and pest control; 10) Detection of latent or obscure pests and pathogens, particularly when first grown; 11) Harvesting, drying, cleaning and inspecting seeds produced by the IARC; and 12) Seed treatment and storage.

The following measures were considered in the review of germplasm exports to evaluate how the IARC's and the quarantine services safeguard the export of germplasm from the IARC to all other countries: 1) Seed treatment before planting; 2) Safeguarding features of the site where material is grown for export; 3) Growing season inspection and pest control; 4) Detection of obscure or latent pests and pathogens in mother plants providing material for export; 5) Harvesting, drying, cleaning and inspection of seeds for export; 6) Seed health testing and/or testing plants grown from samples to detect obscure pests (when applicable); 7) Seed treatment prior to export or storage; 8) Inspection of seeds before export; 9) Receipt of permit from quarantine service of importing country; 10) Phytosanitary certificate issued by the quarantine service of the IARC host country.

To provide a example the following safeguards are used in the movement of cowpea (Vigna unguiculata) by the International Institute of Tropical Agriculture (IITA) in cooperation with the quarantine service of Nigeria in Ibadan, Nigeria.

Imports:

1) A permit issued by Nigeria is required which calls for an added declaration on the phytosanitary certificate that the parent plants were inspected during active growth and found to be free of seed-borne virus diseases; and that either the bacterium Pseudomonas pisi, is unknown in the country of origin or the seeds were harvested from fields which were inspected in active growth and found to be free of P. pisi. 2) A phytosanitary certificate containing the above added declaration issued by the plant quarantine service of the exporting country is required. 3) Seeds are inspected on arrival by the Nigerian plant quarantine service. 4) Seeds are subjected to seed health testing by the moist blotter method. Sprouted seeds which test negatively are transplanted to quarantine greenhouses. 5) Seedlings are observed for symptoms and subject to testing by virus indexing and these eventually produce new seeds. 6) The only seeds released are those harvested from plants produced in steps 4) and 5).

Exports:

1) Seeds (to produce mother plants for growing seed for export) are treated with fungicides before planting. 2) Seeds are sown in the field at IITA during September when there is less risk of mother plants becoming infected with locally occurring seed-borne or aphid-borne viruses than if planted in the dry season when aphid populations are high. 3) Many of the elite lines are resistant to important bacterial and fungal diseases. 4) Plants are insepcted during the growing season by IITA scientists and quarantine inspectors. 5) Plants are tested for viruses by serology and/or indexing. Antisera are available for 6 cowpea viruses and indicator plants for 13 cowpea viruses. 6) The incidence of seed transmission of viruses is determined by an IITA virologist using a 500-seed sample of each lot to be exported. Elite lines with more than 2% seed infection of common viruses are not exported unless authorized on the permit issued by the importing country.

INCREASING SAFEGUARDS AT ORIGIN

Following the trend to emphasize inspection at origin as discussed earlier, the author recommended that quarantine services of importing countries give increased attention to the inspection, detection, treatment and other safeguards as implemented by the IARC's and the quarantine services of their host countries. For quarantine services of importing countries to become more aware of the safeguards and procedures at IARC's, the additional recommendations were: 1) Creation of a new FAO post of plant germplasm quarantine officer who could at the request of an IARC or quarantine service recommend safeguards, provide liaison, and act as a consultant to solve problems related to the timely but safe flow of germplasm. The officer would also provide or locate training for quarantine officers working with germplasm, and provide information on pests and pathogens of quarantine and/or economic significance. 2) For some crops, voluntary use of a plant germplasm health statement by an IARC as an additional document to support the obligatory phytosanitary certificate issued by the quarantine service. The statement would detail safeguarding procedures practised at origin. The statement would be voluntary. 3) Creation of a plant health committee at each IARC to maintain phytosanitary standards for germplasm imports and exports.

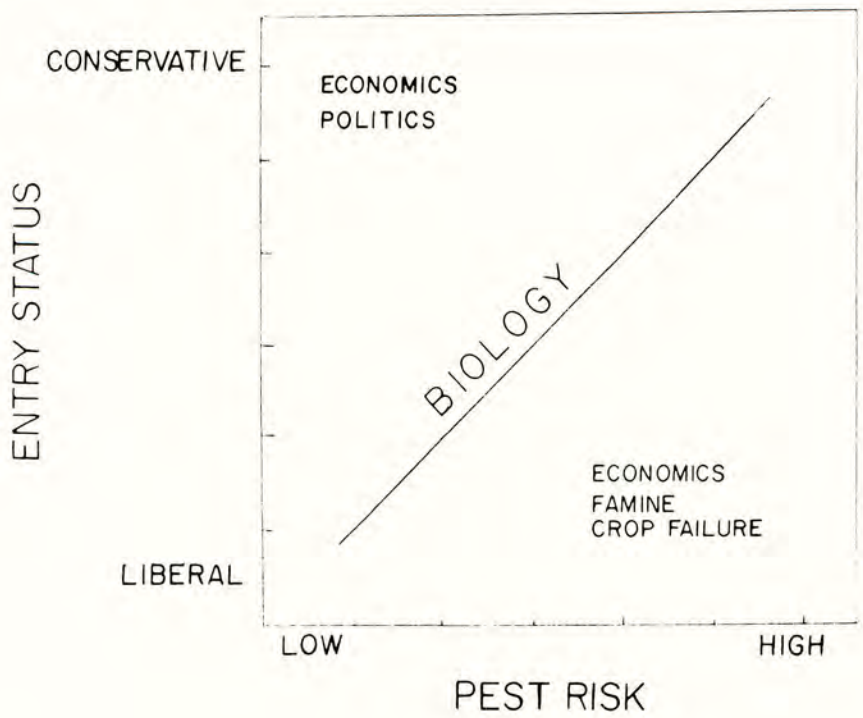

Fig. 1. A diagramatic representation of pest risk analyses. When the actual or perceived pest risk is plotted against the appropriate entry status, a biologically sound match would fall on or near the biological line. Departure from the line indicates the degree of non-biological matching.

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THE RECENT ERADICATION CAMPAIGN AGAINST THE MEDITERRANEAN FRUIT FLY (CERATITIS CAPITATA) IN CALIFORNIA

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Mediterranean fruit fly (Medfly) was first trapped in California on June 5th, 1980. This set the stage for a complex and difficult plant protection programme. From the outset the Federal and State co-operators established the premise that the only acceptable goal was eradication. The technologies employed to achieve eradication, as well as the very concept, generated controversy from within and without the scientific community, with the general public within the affected area, and co-operating agencies.

Initially, control was based on the sterile insect techniques (SIT) with very limited ground pesticide application supported by intensive trapping and regulatory action to prevent artificial spread through the movement of host commodities. As the Santa Clara Valley infestation expanded, the numbers of sterile Medflies released were increased, as was the ground application of pesticides.

When these efforts failed to achieve eradication, aerial application of pesticide was proposed. Resistance and controversy to the programme by the general public within the Santa Clara Valley had been present but limited in degree. A small but voluble minority within the programme area succeeded in blocking efforts to gain required community approvals for aerial application.

Host removal was then undertaken along with SIT as an alternative course over a vast area accompanied by an all out ground pesticide application. Despite these actions, there was conclusive evidence by late June 1981, that the infestation was expanding alarmingly.

During the programme, many States outside of California went to the judicial courts and attempted to restrict movement of California host commodities because of pressure or concern by their agriculture groups. In all cases, restrictions were removed on interstate commodity movement that exceeded the Federal regulations.

The Federal government reacted to the critical nature of the infestation by initiating the preliminary steps to impose a Federal quarantine on host movement throughout the entire State of California. The State then took the necessary emergency action and a co-operative aerial bait spray application was initiated. After aerial bait applications were applied, there was a rapid decline in the infested area and population density, and when the Federal criteria for eradication were met, eradication was declared.

Some countries imposed restrictions at various times during the programme on the importation of Medfly host commodities. The impact of such restrictions on the United States agriculture industry ranged from insignificant to severe, depending on the international market involved.

WORLD DISPERSAL OF SEED-BORNE PATHOGENS

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The scale of dispersal

From 50,000 to 150,000 seed samples are despatched annually by each of several international organisations, including U.N. Food and Agricultural Organisation, Rome; International Rice Research Institute, The Phillipines; Centro Internacional de Mejoramiento de Maiz y Trigo, Mexico; International Crops Research for the Semi-Arid Tropics, India. These seed samples are mostly of cereals, particularly wheat, maize, sorghum and millet, but also include thousands of samples of pigeon pea, chick pea and groundnut. Samples of vegetable and tree seeds are also distributed by professional, commercial and amateur interests. Many germplasm collections, from areas where genetic diversity exists, are distributed to centres in the two or three countries responsible for each particular expedition. In addition, commercial plant breeders exchange samples of cultivars across national and natural barriers for evaluation in different areas. The result is that, each year, "there is no doubt that more than one million seed samples are sent for breeding and trial purposes" (Paul Neergaard, personal communication). Trial grounds provide good "hunting" for pathologists and ensure a diversity of pathogens and strains.

The pathogens that travel

Many pathogens are seed-borne; an individual seed can carry more than one and a sample may contain half-a-dozen different disease organisms. A comprehensive list of seed-borne pathogens required 320 pages including main references in 1979 and a 78 page supplement has already been published (Richardson, 1981). These publications list over fifty fungi on Sorghum spp., six bacteria on Zea mays and twelve viruses from Phaseolus spp. Pathogenic fungi commonly occur on 5-25 per cent of seeds, bacteria around 2 per cent, viruses below 0.5 per cent. Nematodes, often in soil and debris, and insects may also accompany seeds.

Some samples consist of only 200 seeds, more usually they will be of several hundred grams. One international centre recently sent over 600 kg of twenty promising lines or varieties to eight countries in South America; others report sending seed to over 100 countries in a single year. The movement of commercial seed may be more localized but European barley varieties have become popular in New Zealand, Australia and East Africa. Faba beans have been sold not only between European countries but from N. Africa to England and from England to Canada, Australia and New Zealand, successfully transporting pathogenic fungi and viruses.

Potential damage and precautions

The importance of a disease organism to the importing country depends on whether it is already present and on its potential for spread. The latter is difficult to estimate as it depends on variable factors such as weather conditions, varietal susceptibility and the availability and cost of fungicides.

The examination of seed before despatch avoids later rejection (Rohwer 1979). Schemes that issue certificates and labels often include only superficial examination for pathogens. It may be important to discover what is actually done and by whom. The Plant Disease Committee of the International Seed Testing Association compares and verifies laboratory methods and liaises with the Federation Internationale des Semences. Growing-on in quarantine is an effective post-entry method of examination. Chemical treatments are not infallible but do prevent a great deal of harm. Physical treatments (hot water/aerated steam) are equally effective against common and fungicide-resistant strains.

For certain crops the seed-borne pathogens may be so widely dispersed that quarantine action needs to be directed against new pathogenic strains. Simpler methods can protect crops that have received less attention from plant breeders. The further spread of potentially harmful races is greatly reduced when varieties are tested for resistance in a country where the crop is not commercially grown (Ball 1983).

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5C—R2

APHIS (USDA) INTERNATIONAL PROGRAMS

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Background and Objectives

For more than 30 years, the U.S. Department of Agriculture (USDA) has operated several overseas preclearance inspection programs. The object was to have the plant products meet U.S. plant health standards, expedite trade, and reduce costs for the exporter, importer and USDA. This program began in 1951 in The Netherlands with the inspection of flower bulbs before shipment to the U.S. The program expanded to Belgium (1952), France (1956), Italy (1959), Federal Republic of Germany (1964), South Africa (1965), Israel (1972), and England (1980). In addition to the resident officer stationed permanently in The Netherlands, additional Plant Protection and Quarantine (PPQ) Officers have been assigned temporarily to The Netherlands as circumstances and conditions warrant to perform or participate in growing season and export inspection. These have included U.S. supervisory personnel, specialists, and PPQ inspectors working in teams with foreign plant protection officials in the countries involved in the preclearance program.

The success of this program served as a model for others and stimulated PPQ's new International Programs, now being implemented through three international regions.

Ongoing Preclearance Programs

In addition to the flower bulb preclearance program and one for house plants (plants in growing media), begun in 1975, there are now several others:

Chilean fruit - when PPQ began working with Chile some years ago, tons of infested fruits were being sent to the U.S. in the winter season, thus requiring much time and cost in fumigation. With PPQ's technical help, Chile has set up pre-export treatment schedules, and has contacted distributors and growers to eliminate the pest problems at source.

Japanese oranges - for many years Unshu oranges from Japan were prohibited because of a number of pests. Having defined the problems, the Japanese government has taken steps to exclude those pests from orange shipments, which are now subjected to a series of ten different tests, inspections, and diagnostic analyses before export.

Apples and other fruits are inspected for various pests and then placed under cold treatment to eliminate Mediterranean fruit fly larvae in several countries, including France, South Africa, and Australia.

International Programs

The aim is to improve crop protection through increased pest prevention efforts in foreign countries and so facilitate movement of U.S. agricultural imports and exports. In this, the roles of International Programs are to implement all phases of PPQ foreign activities; to provide scientific and technical expertise to U.S. agencies and foreign plant protection organizations wishing to expedite the movement of agricultural exports and imports through improved phytosanitary practices; to provide liaison and to participate with U.S. agencies, specifically the Foreign Agricultural Service (FAS), Department of State, Agency for International Development (AID); national governments; and intergovernmental organizations (such as FAO and regional plant protection organizations) on plant protection and quarantine.

This is to be accomplished through three regional offices, with Region I (Latin America) based in Monterrey, Mexico, with Area Officers in Guatemala City, Guatemala, Lima, Peru, and Santiago, Chile; Region II (Europe, Near East and Africa) in The Hague, Netherlands, and Area Offices in Lisse and The Hague, Netherlands, and Mannheim, Federal Republic of Germany; and Region III (Asia & Pacific) in Agana, Guam temporarily,and an Area Office in Tokyo, Japan. Other Area Offices are to be established within the next three years.

Overseas inspection is advantageous because it permits interception or detection of insects and diseases at source, rather than at U.S. ports of entry, thus reducing the probality of these pests being introduced in the U.S.; more thorough and satisfactory inspections can be made before material is packed; corrective measures to lessen pest risk can be made at the packing houses and growing fields in the country of origin; the plant materials may be shipped to any U.S. destination, whereas in the past, for certain products, only certain specified U.S. ports could be utilized; close collaboration with foreign plant protection services provides monitoring the preclearance system rather than individual inspection at U.S. port of entry; pest conditions can be examined and evaluated in the field, providing for better assessment of current pest situations; and information exchange is greatly facilitated.

PROGRESS IN KEEPING EUROPE FREE FROM ALIEN POTATO PESTS

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EPPO, the European and Mediterranean Plant Protection Organization, is an inter-governmental organization which acts as the regional plant protection organization for Europe and the Mediterranean region, in the sense of the International Plant Protection Convention. With 34 Member Governments, it provides a forum for plant protection services to discuss and cooperate on the measures taken by governments to protect crops and forests, of which one of the most important is the establishment of scientifically based phytosanitary regulations. The approach taken to these is illustrated by reference to progress made in keeping Europe free from alien potato pests.

The EPPO Working Party on Phytosanitary Regulations has first established an "A1" list of non-European quarantine organisms, to be kept out of Europe by a common policy. The organisms on this list include all non-European potato viruses and particularly certain South American potato viruses (Andean potato latent virus, potato virus T, Andean potato mottle virus, etc.). The list also includes certain fungal pathogens (Phoma andina, Angiosorus solani, etc.) and insects and nematodes (Premnotrypes spp., Nacobbus aberrans). A further "A2" list covers quarantine organisms with a limited distribution in Europe, including potato spindle tuber viroid (the main danger of introduction of this organism, and of Corynebacterium sepedonicum, is from North America).

EPPO then recommends that certain regulations should be adopted to exclude all these pests. These regulations include "specific quarantine requirements", made to countries exporting potatoes, or related Solanum spp, to Europe. They distinguish: 1) ware potatoes from America - to be prohibited or treated to prevent germination; 2) seed potatoes from South America - to be prohibited or imported only under strict quarantine conditions, or, from North America, to be prohibited or grown under a rigorous certification scheme for PSTV; 3) material for germplasm conservation, breeding or taxonomic purposes - to be prohibited or imported only under strict quarantine conditions, from any source. These conditions imply: 1) an obligatory license; 2) quarantine procedures in the country of origin; 3) post-entry quarantine.

EPPO then supports these recommendations by publishing data sheets on the organisms concerned (Data Sheets on Quarantine Organisms), with assistance from specialists (in this case, from the International Potato Center in Peru, from the Harpenden Laboratory in England, and from the Biologische Bundesanstalt in FRG). In addition, the specific virological tests (indicators, serology) needed before material can be released from post-entry quarantine are set out in recommended "Quarantine inspection procedures".

Other general recommendations are made, e.g. that true seed or tissue cultures should be preferred for import of germplasm, or that countries should cooperate in providing post-entry quarantine facilities. The ideal is seen to be a very restricted number of points for entry of potato germplasm into Europe.

These recommendations provide a framework for revision and updating of national regulations. Parallel recommendations and publications are made for the other A1 and A2 pests. Cases of special interest, now receiving particular attention, are fruit-tree viruses (linked to national certification procedures), fireblight (the most serious plant quarantine threat in Europe), potato cyst nematodes, and strawberry redcore disease.

5C—R4

THE ELIMINATION OF STRAWBERRY LEAF NEMATODE APHELENCHOIDES FRAGARIAE FROM THE ENGLISH NUCLEAR STOCK PROPAGATION SCHEME

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Background and objectives

The Nuclear Stock Association (NSA) scheme for the vegetative propagation of strawberries of the highest health status is based at National Fruit Trials, Brogdale and at East Malling Research Station. At East Malling mother plants of some 80 varieties, obtained from the original breeders, are maintained. They are tested for freedom from virus and fungus diseases and, each year, twelve or more varieties destined for commercial fruit production are vegetatively propagated before being distributed to specialist propagators for further multiplication under rigorous health conditions. In 1971 some plants of a European variety were introduced into the scheme: they harboured low numbers of the strawberry leaf nematode (Aphelenchoides fragariae) which by 1978 had spread through the contiguous raised beds, in which runners were produced, to many of the other varieties. The infestation was not, at first, noticed because the nematode does not produce symptoms under glasshouse conditions and because nematode sampling, which is destructive, was not then a prerequisite of the scheme. When transferred to the fluctuating temperatures of the field, leaf and crown damage become obvious in some varieties and some stocks were destroyed.

A. fragariae occurs only rarely in British strawberries but is widespread in continental Europe where the serious losses which it could cause are prevented only by regular and continual nematicidal treatment. The NSA scheme was suspended for 2 years while efforts were made to eradicate the nematodes on the premise that if at all possible it should be prevented from becoming endemic in Britain.

Materials and Methods

Several potential therapeutic treatments were investigated: (1) hot water treatment (immersing the plants for 10 min at 46°); (2) chemical treatment with aldicarb, thionazin, oxamyl, thiabendazole or dichlorvos at manufacturers recommended rates; (3) meristem tissue culture and (4) other treatments which had been shown to be promisingly nematicidal in theory but which were thought to be impractical for large-scale commercial use, for example, tissue-disruptive techniques such as ultrasonication or immersion in surfactants or carbohydrates.

Results and conclusions

Although hot water and some of the chemical treatments gave a high degree of control, no technique provided the total control which was essential for the propagation scheme. Even the meristem culture, which has been effective in eradicating virus, bacterial and fungal diseases, was unsuccessful in this case. The nematodes ($< 700\ \mu m$ long x $15\ \mu m$ wide) or their eggs ($70 \times 20\ \mu m$) could easily be transferred to the culture medium along with the c. 1 mm long tissue pieces and 70% of cultures from infested plants were later shown to be themselves infested. However, in the absence of a single effective control measure, tissue culture was chosen as the starting point for a sequence of treatments, as it provided a chance of at least some plants being nematode-free. Thereafter plants were hot-water treated and received applications of aldicarb, and then the mother plants and their runner progeny from separate lines were kept in isolation and subjected to a programme of sampling and rogueing. By these means it was possible to eradicate nematodes from most of the main commercial varieties and in 1982 the scheme was recommenced with the release to the propagators of nine healthy varieties; a further seven varieties were declared healthy in the following year.

The problems posed by this infestation illustrate the difficulty of protecting a vegetative propagation scheme from all possible pests and diseases, and show how vulnerable a single-source scheme can be, no matter how desirable for other reasons. The need for the highest standards of phytosanitation throughout is highlighted by the difficulty of eliminating this pest once established.

MARKETING PLANT DISEASE - CARNATION WILT

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Background and objectives

Fusarium wilt caused by Fusarium oxysporum dianthi is now the greatest single cause of crop reduction and plant loss in carnations grown under glass in the U.K. The disease still develops,even though commercial growers have exploited all types of partial soil sterilisation techniques, and more recently, novel growing systems in isolated beds, peat bags and nutrient film.

Rooted cuttings are supplied by specialist propagators from plant material which may now originate in the Canary Islands, Kenya, Israel, Malta or European countries where mother stocks are retained. British plant health legislation (Statutory Instrument 420, 1980) stipulates that consignments of rooted or unrooted cuttings shall be free from Fusarium oxysporum Schl. Yet, despite growers' efforts to eliminate sources of infection, the first symptoms of wilt can often be detected as early as six weeks after planting.

It was necessary, therefore, to examine planting material delivered from specialist plant propagators in order to establish whether the early disease outbreaks originated from this source, and a summary of this work is presented here.

Materials and Methods

Carnation cuttings rooted in a peat/perlite mixture were sampled on arrival at a grower's nursery. A random sample of cuttings was taken from each box of each variety and the stems cultured on agar to detect the presence of F. ox. dianthi. Peat/perlite from a number of randomly chosen bags each containing 20 cuttings was pelletted and cultured on the selective medium of Komada (1975). The pellet sampling technique was derived from that of Henis et al.(1978). The remaining rooted cuttings were planted in isolated troughs 10 cm deep containing sphagnum peat and irrigated with complete nutrient solution which drained to waste. The incidence of Fusarium wilt was recorded for 18 months from planting in January 1981.

Results and conclusions

F. ox. dianthi was occasionally isolated from the vascular system of 704 rooted carnation cuttings on arrival at the grower's nursery. Tests on the rooting medium, however, revealed the fungus in up to 75% of the boxes tested (15.3% of the 5,790 pellets tested). Disease outbreaks occurred after planting in peat and 41% of the plants were infected after 56 weeks' cropping.

These results are not necessarily typical of all material supplied by carnation cutting producers, but it illustrates the problem of disease transmission with the rooted plant material by a company now operating. Growers depend on the high health standards which propagators aim to maintain; but they have no redress when the growing medium on their own nursery is contaminated and unsuitable for cropping until expensive sterilisation techniques have been used. Pathogen-free propagation material is essential.

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5C—R6

THE COMMERCIAL APPLICATION OF "PATHOGEN-FREE" PRODUCTION SYSTEMS FOR VEGETATIVELY PROPAGATED PLANTS

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The technologies and procedures for indexing vegetatively propagated horticultural plants for systemic pathogens in order to produce propagules which index negatively for such pathogens are well known and continuously being improved. Growing systems for increasing these under conditions which preclude infections by pathogens and infestations by their vectors have also been described. These techniques, procedures and systems have been utilized commercially for many years for the production of what has been termed "pathogen-free" floricultural crops such as Chrysanthemum morifolium, Dianthus caryophyllus and Pelargonium spp.

As disease losses and production costs continue to increase, and as phytosanitary standards continue to improve for vegetatively propagated horticultural crops, the need for and the value of "pathogen-free" propagules of these crops will increase significantly. As the value increases, so will the development of new cost effective indexing and growing systems. This has already happened and will continue to do so with a number of important horticultural crops including begonias, small fruits, roses, several foliage plant species, decidious fruit trees, orchids, grapes and plants grown from bulbs, corms and tubers. There is and will be, therefore, an increasing need and opportunity for commercial ventures.

Success in such commercial ventures over the past 40 years in general, and over the past 10 years in particular, has been mixed, depending upon several major inter-related factors. Among these are:

(1) The implementation of indexing systems that in no way compromise pathological or horticultural quality standards. The indexing system must be cost effective; however, in the balance between "good science" and "good business", the scale must always be weighted in favor of the former and not the latter. Pathologically the key is redundancy to ensure freedom of systemic plant pathogens. Horticulturally, the key is a rigorous breeding and selection program to ensure cultivar quality and stability.

(2) The development of systems that preclude the introduction of pathogens and their vectors under which indexed propagules can be increased to meet market demands. Of critical importance to this is the development of a unidirectional-flow system which is totally renewed annually. Tissue culture provides potential for the increase of indexed propagules, but there are questions concerning cost effectiveness and quality standards.

(3) The development and implementation of new growing systems which will take full advantage of the enhanced potential of the indexed unit and maximize the return on the growers' initial investment. Experience has shown that growers will pay a premium for indexed units; however, continued grower acceptance of this material is dependent upon increased quality and productivity.

(4) An ongoing research and development program. Research and its application is important to the continuous improvement of the total system from the laboratory to the consumer, and, hence, to the long term success of the commercial venture. Research costs, therefore, should in part be borne by the venture as an integral cost of business.

(5) The economics of production and marketing. A commercial venture must obviously function with a reasonable return on its investment, which is dependent in part upon a realistic assessment of the costs of the system relative to its market potential.

An example of the development of a commercial venture which is integrating these factors with some international success is outlined.

THE COCOA SWOLLEN SHOOT VIRUS ERADICATION CAMPAIGN IN GHANA

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Eradication measures are used extensively in attempts to control or at least contain pests or pathogens of diverse crops in many different countries. Plum pox in various parts of northern Europe, citrus tristeza in Israel, sugar cane Fiji in Queensland, banana bunchy top in New South Wales, peach mosaic in U.S.A., little cherry in Canada and coconut cadang cadang in the Philippines are all examples of virus or virus-like diseases of perennial crops that are subject to control by eradication. This is in some instances carried out by government or state employees or enforced by official legislation.

The most ambitious and most expensive eradication campaign every mounted has been against cocoa swollen shoot virus in Ghana, where 'cutting out' measures have been practised on a large scale since the 1940s as the only control measure it has been possible to adopt. The enormous scale of the undertaking is not generally recognised, even though it has from the outset largely monopolised the budget, manpower and resources available for cocoa production and agricultural development in the whole country.

Numerous survey parties are employed by government or quasi-government agencies to carry out periodic inspections of all cocoa-growing areas. Outbreaks are then treated and retreated as necessary by cutting out all visibly infected trees. Official compensation is paid to growers for the loss of trees and there is also a replanting grant or treated farms are replanted before being handed back to the original owners. These measures were originally enforced but they are now operated on a voluntary basis and for the last 3 years have been practised on a very limited scale.

Collated data are available up to the end of 1982, by which time 185.5 million infected trees had been eradicated and 64% of these were removed in carrying out the initial treatment of newly discovered outbreaks. The number of trees destroyed is equivalent to 123,000 ha at usual spacings and excludes the many millions of trees killed by swollen shoot before they were found by the inspectors.

The eradication campaign has been fully justified and is reasonably successful in five of the six main cocoa growing regions, where almost all the known outbreaks have been treated and where the number of trees destroyed (17.0 million) is small in relation to the total tree population and to the value of the cocoa produced. The situation is very different in Eastern Region where swollen shoot was first discovered in 1936 and where infection is now rife in many areas. The number of trees eradicated since 1945 totals 168.5 million, yet it is known that there was a backlog of 31.2 million infected trees to be removed in 1982. The true situation is likely to be far worse because there must have been much further spread since the last comprehensive survey was carried out. Moreover, recent studies have shown that the survey parties find only about 23% of all the infected trees in a new outbreak because many are missed or in the latent phase of infection. The situation is obviously unsatisfactory and is likely to deteriorate further because only about a million trees a year are being eradicated in the current phase of the campaign.

The failure of the eradication policy in the Eastern Region is only partly due to the sheer magnitude of the problem and to the difficulties of organising and supervising such a major undertaking. There has been a lack of continuity in the campaign and much effort has been dissipated in treating and replanting individual farms that are often small and surrounded by untreated or abandoned cocoa containing numerous sources of infection. Reinfection is inevitable in these circumstances and often occurs at an early stage so that many of the affected farms are young and not yet in full production. A reassessment of current procedures in the Eastern Region is long overdue and there is an urgent need for epidemiology studies to determine safe isolation distances, to assess the merits of treating large contiguous blocks and to find methods of deploying to best advantage the resistant varieties now available. It should eventually be possible to develop improved methods of treating and replanting affected areas in such a way that there is little serious risk of reinfection.

5C—R8

THE USE OF SYNTHETIC SEX PHEROMONES IN PLANT QUARANTINE IN THE USSR

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In recent years, pheromones have been used to detect the Oriental fruit moth (Grapholitha molesta Busck.), potato moth (Phthorimaea operculella Zell), San Jose scale (Quadraspidiotus perniciosus Comst.) and Comstock mealybug (Pseudococcus Komstocki Kuw.).

Sticky traps with a three-component pheromone (ZT and 8-DDA + Dodecanol) are used to detect Gr. molesta. About 50,000 traps are used annually. Technology for the control of this moth by disorientation has been developed, using special pheromone formulations. Samples of a highly specific pheromone have been obtained which does not attract males of the plum fruit moth, Gr. funebrana, thus making analysis of the genetalia unnecessary for identification.

The attractiveness of the synthetic sex pheromone is different for males of spring and summer generations. The pheromone shows high specificity and does not attract males of other closely related species. Studies of diurnal rhythms in the activity of the San Jose scale in the Northern Caucasus and Crimea showed that peak flight occurred from 1800 to 2100 h at 25°C. The number of males trapped depends on population densities and distance from infested trees. Thus, trap catches were about 3,000 and 100 males at densities of 12 and 0.2 insects/cm^2, respectively. One trap per 2 ha is sufficient to survey nurseries for San Jose scale.

Potato moth pheromone (a single-component composition RTM-I) is widely used in the USSR to detect infestations of the pest using c. 30,000 traps. Tests with the second component (RTM-II) and a mixture of II and I in the ratio 1:4 showed higher attractivity.

Further expansion of research and the practical application of insect pheromones will soon make it possible to completely replace visual examinations by highly efficient specific methods based on chemicals. It will greatly increase the reliability of detecting new foci of infestation of quarantine pests and will substantially reduce survey costs.

THE ASSOCIATION OF ASPARAGUS VIRUS 2 AND FUSARIA IN ASPARAGUS SEED

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Background and objectives

Analysis of seed samples of *Asparagus officinalis* L. cv Precoce di Argenteuil - widely used in Emilia-Romagna region - frequently revealed Asparagus virus 2 (AV2) and *Fusarium* spp. Forty percent of the seeds obtained from virus-diseased plants was infected by AV2. *Fusarium* spp was noted on both captan-treated and untreated commercial seed-samples. *F. oxysporum* and *F. moniliforme* were very frequent, whereas *F. roseum* var. *culmorum* was relatively rare. The aim of the present work was to determine the importance of AV2 and *Fusaria* both separately and in mixed infections.

Material and Methods

Two commercial samples taken from *Fusarium* infected fields were used. Each sample contained 500 seeds. Two-three month old plants were tested for the presence of AV2 with mechanical inoculation indicator plants. Seeds infected with *Fusaria* were treated following the Damicone and Cooley method.

This method was used on both small (15 g) and commercial (7-8 kg) lots. Afterwards AV2-infected seedlings were inoculated with spore-suspensions of two *F. oxysporum* strains of known pathogenecity.

Results and conclusions

The infection of AV2 in the commercial seed-lots varied from 10% to 60%. These findings are in good agreement with the literature. The method elaborated by Damicone and Cooley provided a good infection control not only on small samples but even on the commercial batches. The first results with virus-fungus mixed infection suggest that the debilitating effect of AV2 (which alone causes a yield reduction of 8%) is increased by the action of *F. oxysporum* and *F. moniliforme* in the soil, especially during the first year. It seems very likely that even a low potential inoculum of *Fusarium* in the soil can cause the "decline" of Asparagus plants when AV2 infected material is used for multiplication.

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