Healthy Planting Material: Strategies and Technologies

BCPC Monograph No. 33

Preface

The need to ensure healthy planting material for vegetatively propagated crops has long been a requirement whether it be seed potatoes, strawberry runners or dry bulbs intended for export. Troublesome pests and diseases range from nematodes, blackleg and viral diseases in potatoes to *Verticillium* wilt in hops and viral and fungal diseases in bulbs and flower crops. Freedom from viral disease is especially important in fruit and ornamental crops and has led to the establishment of nuclear stock schemes for the production and distribution of virus-tested and virus-free stocks. Seed-borne diseases may also pose problems for farmers and growers but control will generally be achieved by chemical treatments.

Over the past decade there has been a rapid development of techniques for the identification and characterisation of disease pathogens, and for the production of healthy planting material involving tissue culture and micro-propagation. These newer techniques have facilitated the improvement of existing certification schemes and the introduction of new ones, and have helped growers to meet the exacting requirements of export and import control measures and quarantine. These developments have been timely because a new industry has sprung up devoted to the rapid propagation of plants to meet the demands of international markets.

The present Symposium represented something of a departure for the British Crop Protection Council from topics principally concerned with the use of pesticides. It was conceived jointly with the Association of Applied Biologists to provide a wide-ranging review of the strategies and technologies of certification and cleanstock schemes, and of the viral, bacterial and fungal pathogens and pests that have to be eliminated. One session is devoted to the use of pesticides and the final session to the new technologies.

I should like to thank all those who contributed to the Symposium, particularly the Programme Committee, and especially my colleague and Vice Chairman, Dr Allen Langton.

D. RUDD-JONES

Symposium Programme Committee

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Discussion			
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Scope of the Symposium

The Symposium brought research workers, advisers and growers concerned with the need to produce disease- and pest-free planting material and to develop treatments to ensure continued plant health. Its scope extends across all vegetatively propagated and seed-raised temperate crops to generate a wide-ranging appreciation of what can be done and what will become possible in the future.

Plenary lectures discuss past achievements, current needs and future opportunities. Invited speakers consider certification aspects and clean-stock schemes, particular problems and solutions relating to viral, fungal and bacterial infection, pest problems and the use of pesticides as seed treatments, and the role of micropropagation and advanced methods of disease detection. Poster displays formed an integral part of the meeting, demonstrating current work in a wide range of associated disciplines.

Acknowledgements

The following kindly contributed towards the cost of the reception:

Imperial Chemical Industries PLC, Plant Protection Division,
Jealott's Hill Research Station, Bracknell, Berks RG12 6EY

BASF United Kingdom Ltd
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ABBREVIATIONS

acid equivalent	a.e.	nuclear magnetic resonance	nmr
active ingredient	a.i.	number average diameter	n.a.d.
aqueous concentrate	a.c.	number median diameter	n.m.d.
boiling point	b.p.	organic matter	o.m.
British Standards Institution	BSI	page	p.
centimetre(s)	cm	pages	pp.
concentration	concn	parts per million by volume	mg/l
concentration × time product	ct	parts per million by weight	mg/kg
concentration required to kill 50% of test organisms	LC50	pascal	Pa
correlation coefficient	*r*	percentage	%
cultivar	cv.	post-emergence	post-em.
cultivars	cvs.	power take off	p.t.o.
day(s)	d	pre-emergence	pre-em.
days after treatment	DAT	probability (statistical)	*P*
degrees Celsius (centigrade)	°C	relative humidity	r.h.
dose required to kill 50% of test organisms	LD50	revolutions per minute	rev/min
dry matter	d.m.	second (time unit)	s
Edition	Edn	standard error	S.E.
Editor	Ed.	standard error of means	S.E.M.
Editors	Eds	soluble powder	s.p.
emulsifiable concentrate	e.c.	species (singular)	sp.
freezing point	f.p.	species (plural)	spp.
gas chromatography-mass spectrometry	g.c.m.s.	square metre	m^2
gas-liquid chromatography	g.l.c.	subspecies	ssp.
gram(s)	g	surface mean diameter	s.m.d.
growth stage	GS	suspension concentrate	s.c.
hectare(s)	ha	temperature	temp.
high performance (or pressure) liquid chromatography	h.p.l.c.	thin-layer chromatography	t.l.c.
hour	h	tonne(s)	t
infrared	i.r.	ultraviolet	u.v.
International Standardisation Organisation	ISO	vapour pressure	v.p.
Kelvin	K	variety (wild plant use)	var.
kilogram(s)	kg	volume	*V*
least significant difference	L.S.D.	volume median diameter of drop spray	v.a.d.
litre(s)	litre	weight	wt
litres per hectare	l/ha	weight by volume	wt/*V*
mass	*m*	(mass by volume is more correct)	(m*V*/)
mass per mass	*m*/*m*	weight by weight	wt/wt
mass per volume	*m*/*V*	(mass by mass is more correct)	*(m/m)*
mass spectrometry	m.s.	wettable powder	w.p.
maximum	max.		
melting point	m.p.	approximately	≃
metre(s)	m	less than	<
milligram(s)	mg	more than	>
millilitre(s)	ml	not less than	≮
millimetre(s)	mm	not more than	≯
minimum	min.	Multiplying symbols—	Prefixes
minute (time unit)	min	mega ($\times 10^6$)	M
molar concentration	M	kilo ($\times 10^3$)	k
		milli ($\times 10^{-3}$)	m
		micro ($\times 10^{-6}$)	μ
		nano ($\times 10^{-9}$)	n
		pico ($\times 10^{-12}$)	p

Plenary Lectures

Session Organiser : D. Rudd-Jones

1986 BCPC MONO. No. 33 SYMPOSIUM ON HEALTHY PLANTING MATERIAL

HEALTHY PLANTING MATERIAL: ACHIEVEMENTS, NEEDS AND OPPORTUNITIES

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INTRODUCTION

The full importance of crop production as the key component in meeting the increasing world requirements for food, fodder, animal feed, renewable fuel resources, pharmaceutical and chemical feed stocks and in ecological and amenity improvement has become more clearly recognised in recent years.

Some estimates of projected human population by the end of the century now exceed 7,000 million and the essential food requirement, combined with higher dietary expectations and emerging nutritional and medical doubts about specific animal products has placed an increased emphasis on foods directly obtained from plants. This will demand improvements in crop productivity, quality and diversity on a world-wide basis notwithstanding the distortions resulting from the current limited agricultural surpluses in certain products in some regions, particularly the European Economic Community and the United States of America.

The role of plants in maintaining environmental balances and their influence on climates is, at last, more widely appreciated (helped by the recent public interest in drought and famine situations). Possibilities for reclamation and realistic re-afforestation projects are emerging. More opportunities to develop the potential of plants for biomass production and for more highly specific and for general uses are being identified. It is therefore appropriate that this Symposium, covering all aspects of health of the planting materials required to meet this expanding demand for temperate crop species, should be taking place and encompassing differing aspects and many disciplines. The subjects, covered both in seed production and vegetative propagation, although concentrating upon temperate crops, have a direct relevance to tropical crops and experiences gained with the latter have applications to temperate crops. This first plenary paper will cover some of the principles underlying the many technical and organizational factors involved; all commercial considerations will be left to the second paper.

The use of appropriate planting material has long been recognised as an important, or even the key element, in growing successfully either individual plants or crops. It is, perhaps, now self-evident that if poor or wrong planting material is used all later cultural operations will be adversely affected. However, such material is still widely used. An appreciation of the advantages of the correct choice, or deliberate selection, of superior planting material stems back to the earliest days of settled agriculture. There is good evidence for this from many cultivated species themselves. For example, the evolution of bread wheat (Triticum aestivum) as a hexaploid species was advanced by human conservation, protection and active selection. Similar trends in the evolutionary processes are seen in other long-established horticultural and agricultural species such as roses, vines, peaches and potatoes.

Man's desire to improve plants was recorded in early writings. The Romans were very active in this field and Pliny, in the first century A.D., referring to the contemporary craze for bigger and better vegetables wrote "... there is nothing that pleases man in the fashion (of the plant) in

which Nature finally made it". The process of 'improvement' by selection, in crops as diverse as fruits and cereals in the old world or corn (Zea mais) and potatoes (Solanum tuberosum) in the new, was practised everywhere. Culling became established in the traditions of the specialized plant-collectors and selectors and early 'scientific' plant breeders in Europe from the sixteenth to the beginning of the twentieth century. Modern plant breeding combines selection with controlled hybridization or other techniques to generate genetic diversity.

The objectives of this 'improvement' are easily understandable. What is not clear is when, and to what extent, three essential features of propagating material, its 'heredity' (genetic composition), its nutrition and its health, were recognised in principle if not in the precise terms we understand today. It may well have been earlier than previously believed. However, by the beginning of the twentieth century the importance of the 'constitution' and health of planting material, including propagation stocks, and the need for such material to be well-documented, available and widely used was accepted in Europe and the United States of America. In the United Kingdom the National Institute of Agricultural Botany was established in 1920 with the motto 'Better Seeds - Better Crops' and although the meaning of 'better' was not defined it was generally understood. Comparable bodies were set up in many other countries. Since that time the advantages and benefits of using good planting material have been amply demonstrated and much has been learned about the practice and scientific basis of its production. The year 1961 was designated by The United Nations Food and Agriculture Organization (F.A.O.) as 'Seeds Year' and much publicity was given world-wide to the importance of healthy planting material.

Present levels of crop productivity depend heavily upon established plant breeding, seed production and propagation technologies. Although ultimate yield and quality are determined in the growing phase by other inputs and technologies - fertilizer, disease, pest and weed control, irrigation, engineering etc. such contributions are only fully utilized if the genetic composition and health status of the planting material is adequate. All crop production incurs many hazards; the use of healthy propagating material reduces some of the risks involved.

Many opportunities exist for the wider application of current knowledge either by simple modifications to the practices involved, or by extending the range of crops to which they are applied. New developments now allow improvements to be made to establish production procedures for both seed and vegetatively propagated stocks and in some cases will revolutionize whole production systems. Tissue culture techniques have been rapidly exploited to improve the health status and increase the scale of vegetative propagation processes. This may be combined with cloning to improve the genetic uniformity of varieties of out-pollinating species, particularly vegetables. Changes introduced to breeding methods such as the application of the F1 hybrid system to self-pollinating species have led to new seed production systems. In some cases developments in both areas have occurred almost simultaneously. For example, commercial quantities of F1 hybrid tomato plants, grown from a few plants raised from few seeds and subsequently cloned can change a traditional 'true seed' glasshouse production system to one based upon vegetative planting material. The cost is similar and gives the advantages of absolute genetic unformity, greater reliability of establishment and a more controllable and predictable

harvest. Similar procedures will encourage the exploitation of heterosis in crops where F1 hybrid seed is difficult or expensive to produce.

BENEFITS OF HEALTH

It is impossible to attempt to quantify reliably the losses that can result from the use of unhealthy propagating material; it is very difficult to collect accurate data on losses due to single pests or diseases in every crop. Losses in production are frequently the result of the effects of several pathogenic agents acting simultaneously, or in sequence, and may be either direct or indirect, or both.

Direct losses may range from total crop failure to marginal yield reductions or to damage to end products which may be devalued by quality defects or be themselves diseased. Examples of such losses can be seen, for example, in potatoes where, even with extensive hygiene and certification control of planting material, the combination of tuber transmitted bacterial and fungal diseases (blackleg, gangrene etc.), viruses (PLR, PVX and PVY) and nematodes, crop failures in the field may be total, or post-harvest losses in store may be severe. In contrast, the reduction in yield of wheat or barley through use of seed infected with loose smut (Ustilago spp.) is usually trivial but, re-infection by the fungus at flowering time can easily be sufficient to cause rejection of the resultant crop as seed (with the financial penalties of the loss of the seed premium or incurring additional administrative and seed-testing burdens and the on-cost of chemically treating the seed).

Extensive indirect losses can occur through the introduction of disease into stands of otherwise healthy crops by the use of infected replant material e.g. virus infections in soft fruit plantations.

The elimination, or reduction, of infected or infested planting material by the techniques currently in use such as visual and microscopic examination, virus screening or chemical treatment, often backed-up by certification, can be very successful. However, such controls are not universal or can be avoided and require a degree of sophistication in both national and international trade. If control procedures become less stringent through, for example, a large increase in the volume of material or cost cutting exercises by administrative authorities, evasion may increase with inevitable consequences. Another risk may arise if hygiene procedures are not assiduously applied to material produced by 'novel' techniques. There is great mobility of propagating material made possible by the high value of elite materials and by ease of packaging and air transport. The potential to reduce losses still further exists but so does the possibility of increasing losses through dangers arising from the distribution, perhaps unknowingly, of unsatisfactory material more widely and thus introducing pests and diseases into new areas.

High standards of hygiene and health are, therefore, essential and must be established and maintained either by commercial competition or by certification or by a combination of both.

THE PRESENT POSITION

True Seed

The principles and practices of production of 'true seed' have been refined over the years and, although requiring constant review to take account of changing biological situations, such as disease spectra,

adequate amounts of high quality seed can usually be produced. Unfavourable weather does occasionally reduce both the quantity, and the germinability, of seed as well as adversely affecting other characteristics of quality. To some extent this difficulty has been overcome by the greater internationalization of seed production in recent years.

However, some demands of present day practice may become increasingly difficult to meet in the future. Seed, like other multiplication material, has to meet rigorous standards of genetic uniformity (varietal and specific purity), performance, health, and freedom from weeds. In the U.K., and in many other countries, the production of seed of the major agricultural and horticultural crop species is controlled by exacting official, or voluntary, certification procedures. Rigid crop rotational requirements for land used for seed production and pre- and post-harvest crop inspections are effective but are restrictive, labour intensive, and costly. Suitable land for seed production is increasingly difficult to find. The harmonization of seed certification and testing regulations between countries, particularly in the E.E.C., is proceeding and will simplify international trading in seed which can relieve land shortage or climatic problems. It is to be hoped that expenditure cuts by governments will not cause a delay in the implementation of the more co-ordinated regulations. However, consciousness by the growers of the need to lessen production costs, particularly in relation to the avoidance of losses through disease must increase the importance and value of healthy seed. Seed, of every sort, will inevitably become a more expensive commodity. The application of new methodology, such as electrophoresis for varietal identification checks, and other tests for viability, vigour and absence of disease may help to keep costs in check but good quality seed is already a high value product although, in many cases, seed is only a small part of the input cost of modern crop production.

The health status of seed has, for many years, been enhanced by physical and chemical treatments. Mechanical screening eliminates much unhealthy or potentially dangerous material such as weak seed and debris. Rubbing (to separate seed clusters) and pelletting can further improve the product. Heat, hot water and chemical soaking treatments have been used to reduce some internal infections. Mercurial compounds and other chemicals have been very effective in eliminating externally carried pathogens, for example, bunt in wheat (Tilletia caries) or fungi which adversely affect seedling establishment. Other attempts to protect seed were not really effective until the position changed rapidly with the introduction of systemic fungicides and new seed coating techniques. Seed itself can now be safeguarded against many hazards and it can also be a highly efficient carrier for agri-chemicals of various sorts, to protect and improve the later growth of the crop.

Further evolution of this type of technology can be expected to lead to advances in several directions including the incorporation and more precise placing of more complex chemical combinations giving prolonged fungicide and pesticide protection. It will be particularly valuable if the elusive, downwardly translocated, fungicides can eventually be developed.

Bulbs and tubers

The bulky nature of bulbs and tubers combined with the soil which adheres to them gives protection to pathogens and provides ideal conditions for the survival and transmission of pests and diseases.

Improvement in the health status of bulbs (and similar propagating

material) has been one of the most spectacular achievements in the post-war period. Progress has been steady in the elimination of virus-infections in stocks by various techniques, in reduction of the incidence of fungal diseases and in methods of multiplication. The availability of healthy stocks has transformed the cultivation, and the commercial value, of some species. The improvement is sustained by certification in some species and by chemical treatments.

Potatoes present quite a different situation. Ware growing, at present, is dependent upon conventional seed-tuber production and the "seed" is prone to attack by many pathogens both in the field and in store. Even when "clean stocks" are produced the slow rate of multiplication over several years gives many opportunities for re-infection before "seed" reaches the grower. Although the development of the VTSC system has proved very successful it has to be supported by an extensive and expensive certification system, by the designation of high-quality seed production areas and statutory movement control, all under a chemical "umbrella". Present developments in tissue culture and the production of mini-tubers offer possibilities of improving this system. These techniques also offer to breeders the opportunity of accelerating the multiplication stage of new varieties for assessment, testing and introduction. It now takes about four years to multiply, in isolation areas, stocks of new varieties up to the 6-7000 tuber stage (when independent testing can commence) with the hazard of re-infection by disease. This can be reduced to about six months using in vitro aseptic techniques. The prospect of using disease-free, mini-tubers transplanted in peat blocks in production systems in the U.K., or in warmer climates (where storage of conventional tubers is extremely difficult) is now opening up at least for final "seed" multiplication - if not for ware production.

Seedlings and plantlets

Because of their vulnerability, seedlings and cuttings have, in the past, only been a very small component of the output of the propagation industry and have been largely confined to decorative species or to special programmes (e.g. seed production for biennials such as sugar beet).

The position is now rapidly changing with the introduction of plantlets and convenient peat block transplant systems. These systems have commercial attractions making good use of expensive seed and of rapid multiplication techniques to produce uniform, vigorous, disease-free transplants. However, there are also dangers involved. Genetic variants may occur and if, for any reason, health control in propagation units fails, diseases can be disseminated widely and rapidly. For instance, there is a possible danger of this type from big-vein virus in transplants of F1 hybrid lettuce and also from chrysanthemum white rust (Puccinia horiana). Once again the importance of health control and monitoring of disease, or incipient disease, is emphasized.

Although new developments always attract attention an example of the need for vigilance with more conventional seedlings for propagation has recently occurred. The appearance of 'Rizomania' in sugar beet in Europe led, in 1984, to the introduction of new quarantine regulations in the U.K. concerning import of beet seedlings and debris which have so far been successful in excluding this complex disease.

The influence of plant breeding

This Symposium must not ignore the continuing progress in plant breeding, although a great deal of time cannot be devoted to it. The

impact of breeding is twofold. First it provides new varieties and secondly, it introduces new types of material to be multiplied. In the recent past breeders, largely using conventional methods, have provided streams of 'improved' varieties of established crops and brought into production additional species including, in the U.K., oil seed rape (Brassica napus), Triticale and many types of decorative plants. The improvements made by breeders affect many characteristics of the plants including disease and pest resistance, plant architecture and physiology (to meet changing crop husbandry needs) and product quality as well as yield.

Genetic resistance is the first line of defence against fungi, pests, viruses and nematodes. Inbuilt resistance despite its transient nature due to pathogen variability, is very cost effective and environmentally acceptable and it is a prime objective of many breeding programmes. Change in pathogen virulence is a major factor in eliminating varieties from cultivation and indirectly is responsible for the plethora of varieties which so complicates multiplication programmes.

If breeders incorporate into their varieties disease and pest resistance to pathogens which affect seed or propagating material, the task of the multiplier is made easier but breeders do not always focus on such objectives. In many cases breeders do not have access to sources of resistance to such pathogens or if they are available they are difficult to use.

There seems to be every reason to expect that breeders will continue to innovate and develop new varieties, probably at an increasing rate, using both conventional techniques and those resulting from the advances being made in genetic engineering.

Plant breeders, by adopting the F1 hybrid system for more crops and by experimenting with apomixis are providing challenges to seed production technologists and giving opportunities to develop healthier seed. In contrast, some of the changes bring additional hazards such as the 'Texas' cytoplasm problem in 1969/71 with F1 hybrid corn and race T of Drechslera maydis and ergot (Claviceps purpurea) infection in F1 hybrid wheat.

Efficient exploitation of the new varieties, whichever way they are bred, will remain dependent upon the rapid multiplication of healthy seed or propagating materials. If there is a delay much of the benefit of plant breeding may be lost. Traditional breeding organizations often devote up to one-half of their effort to various aspects of 'maintenance breeding' in order to ensure a continuing supply of highly uniform multiplication material to satisfy statutory requirements. These programmes by the breeders are supplemented in the U.K. by the work of bodies such as the nuclear stock associations and other producers of multiplication material e.g. virus tested 'seed' of potatoes.

A current trend in plant breeding which affects multiplication is to accede to increasing pressure by statutory authorities for higher levels of genetic uniformity in varieties. There is an increased danger to genetically homogeneous varieties from pathogens. The drive for uniformity can easily be understood in order to satisfy consumer protection requirements and for the administration of Plant Breeders Rights. Extreme uniformity runs counter to the position which occurs in nature - and offends commonsense. Natural breeding systems have evolved in various ways

to achieve and sustain genetic diversity. Current developments in systems developed by breeders may increase genetic uniformity still further as will clonal propagation techniques. It, therefore, becomes important to recognise this danger and to consider alternative strategies to restore some degree of diversity.

Protection of intellectual property rights

The speed of absorption of new technology into plant breeding and production systems will obviously depend upon the level of research activity and the relevance of the results to development programmes. At a time of reduced government funding in many countries, costs of research and development will increasingly have to be met by the private sector. Organisations involved in the pioneering of new techniques will be seeking to generate funds, by commercial exploitation of 'know-how' as well as by the sale of products, to maintain the momentum of the increasingly expensive research.

The satisfactory protection of all intellectual property rights is, therefore, of very considerable importance. It is unlikely that 'in-house secrecy' will be a satisfactory solution in the longer-term, although it will have a role. Patent protection may be appropriate for processes and even for some end products. Patenting and, in some countries, Plant Breeders Rights (PBR) are available for the protection of reproductive material of higher plants. The protection of genetic material is already complex internationally because of differing national laws for PBR and is further confused as both the type, and the degree, of protection conferred varies between the patent and the PBR pathways.

Plant Breeders Rights in countries where they are operative, impinge upon multiplication techniques because propagation utilizes reproductive material which is the exclusive right of the owner. The term 'reproductive material' requires very careful definition as almost any plant component can now be considered as reproductive. Large scale propagation can also result in the occasional appearance of unique genetic material as off-types. Such variants, whose origins cannot always be fully explained, could have commercial value as new varieties in their own right. The ownership of the new varieties arising in this way may be agreed by pre-emptive contractual agreements between the owners of the original variety and the propagator. If such arrangements are not made, the legal ownership of the new variety is uncertain even where PBR exist and when the genetic differences are sufficient to satisfy the distinctness requirements for a grant of Rights.

PBR legislation is controlled by various international agreements and understandings. It is subject to guidelines promulgated by the International Union for the Protection of New Varieties of Plants (UPOV), an offshoot of the World Intellectual Property Organization (WIPO) of the United Nations. Protection is given to 'reproductive material' which is defined in various ways in national law. The UPOV system has not been universally accepted and now applies only in the 17 member countries of UPOV (see footnote). The principle of PBR is opposed by some countries and

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Belgium	Ireland	New Zealand	United Kingdom
Denmark	Israel	South Africa	United States of America
France	Italy	Spain	
Germany (FDR)	Japan	Sweden	
Hungary	Netherlands	Switzerland	

by some international organizations and pressure groups. Objections are based upon moral and practical grounds and consequently, PVR are unlikely to be introduced much more widely in the immediate future. Indeed, even in countries where PBR are established, criticism is intensifying because of the cost of operating the system and of technical difficulties which have emerged. The expense of implementation (through an independent testing system to meet the requirements of distinctness, uniformity and stability) is already unjustifiably high and will rapidly increase as the tests become more elaborate and differences between candidate varieties (minimal distances) become less. The PBR system itself is slow and is insufficiently flexible to meet the needs of breeders of some species, particularly decoratives and some vegetables, where varieties (subject to rapid changes in fashion) are many in number and have a short life. In the U.K. the Plant Variety and Seeds Act became law in 1964. It has since been updated by various amendments culminating in major changes introduced in the Plant Varieties Act 1983. The legislation must soon be due for Parliamentary review to determine if it has achieved its original object and is continuing to serve the public interest. It may well be found that it has failed, or that it has become costly and inefficient. In any event, the interaction and overlap of PBR and Patent protection needs examination in several areas and most particularly in relation to plant breeding processes and to the protection of the techniques and products of genetic engineering. It is now conceivable that a new variety may be protected by PBR but also contain patented components. International discussions on these matters are already taking place but it is unclear how, or when, the situation will be resolved but complete reliance upon patents and the abandonment of PBR is one possible solution. The intricacies cannot be discussed at length here but possible legal obstacles detracting from the successful commercial exploitation of research should not be underestimated. It is essential that the position should be clarified if future venture capital investment in research in this field is to be encouraged and rewarded.

FUTURE OPPORTUNITIES

It is the objective of this Symposium to generate a greater awareness of what can be done, or what might become possible in the future, to produce healthier crops. It is important to define two distinct time-scales, the almost immediate future and a longer-term covering approximately twenty years.

Immediately, further substantial improvements in crop health can be achieved by extending the application of existing, or slightly modified, technologies to a greater volume of material, to more species and to more countries. This is largely a matter of commercial development, information transfer and advisory work. At a time of no overall food shortage the limitation of progress is unfortunately due more to a shortage of money (for some research and development and within the industry) and a lack of incentive and will than to an inadequacy of scientific information.

In the longer-term, in twenty years time, there may well be an absolute shortage of food. When this appears to be imminent the minds of politicians will turn towards agriculture and horticulture and there will be pressure to increase production rapidly. However, as has happened in the past, predictions of food shortages have been confounded by steady advances in technology and production and thus a change in the economic scenario cannot be assumed.

In this situation what opportunities might be highlighted by this Symposium? The following are possible areas:

1. New diagnostic techniques. Healthy stock propagation systems depend upon techniques for the identification of "clean" base material. Advances in the development of DNA probes will allow the detection of many viruses and viroids (e.g. PLR and potato spindle tuber viroid - PSTV) very precisely and at low levels.

2. New disease resistant varieties. New techniques, including protoplast-fusion and other genetic engineering methodology may enable wider and more stable ranges of disease and pest resistance to be identified and introduced.

3. Wider use of clonal propagation techniques. Application of these techniques to a broader range of 'cleaned-up' material will provide superior elite stocks of many more species.

4. Greater internationalisation. Provided that the opportunity is taken to develop appropriate quarantine regulations and import/export understandings, benefits will accrue from international co-operation and competition. Opportunities will occur for the production in the U.K. of propagating material of both indigenous and exotic species for home use and for export. This will involve developing appropriate handling techniques and a capacity to test for freedom from an extended range of pathogens.

5. Replacement of established husbandry systems. Possibilities of substantial changes in husbandry methods are emerging. These could range from major economies in seed use (by preparation of protected seed propagules) to direct use by the grower of plantlets rather than seed. Such healthy planting material will increasingly become associated with integrated disease control (and disease avoidance) strategies. More esoteric possibilities might be changes from vegetative reproduction to use of true seed as, for example, in potatoes or vice versa. The speed of introduction of any such changes may be very rapid.

In the papers which follow, various aspects of these possibilities, and many others, will be discussed. The way in which healthy propagating material is produced, becomes available, is multiplied and presented to the grower will change.

Technical innovation has revolutionized many areas of plant multiplication in recent years. This field will probably be the first in crop production to benefit at a practical level from the integration of both 'low' and 'high' level biotechnology with traditional systems. The challenges and opportunities offer a fascinating and rewarding future to all sectors of the industry but it is a future in which many long-established and well-proven practices will need to be revised.

COMMERCIAL OPPORTUNITIES FOR TISSUE CULTURED DISEASE INDEXED PLANTING MATERIAL

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INTRODUCTION

Systemic pathogens cause large crop losses but schemes to produce clean planting stock have been developed for a number of crops. This paper draws attention to the opportunities resulting from use of tissue culture techniques in the commercial production of high health planting material.

FEATURES OF SYSTEMIC PATHOGENS

Farmers and growers throughout the world depend upon the plant breeding industry to develop improved varieties so that production may be increased. The genetic gain captured by breeders which has led to some remarkable yield increases is eroded by attack from pests and diseases. This loss can be countered by use of agrochemicals and by breeding for pest and disease resistance.

In the case of disease caused by systemic pathogens, which invade the vascular system and enter host plant cells, agrochemicals are of little use. They cannot adequately be targeted at a pathogen inside the plant. If resistance genes are unavailable in crop varieties then alternative methods of control must be sought.

Methods will vary depending on the crop. In general, true seed is free from systemic pathogens and crops become infected through the agency of vectors which may be insects or soil inhabiting organisms. Provided seed is properly tested, each crop starts off "clean" and control measures can therefore be aimed at vectors.

The situation is very different in the case of vegetatively propagated crops. These are no less vulnerable to infection through the agency of a vector but when the plant is propagated, so is any pathogen. By the repeated process of acquisition and propagation vegetatively propagated plants can become increasingly "loaded" with systemic pathogens with consequent effects on yield. A graphic example of this is shown by the work of Kolbe (1981) in which the yield of replanted potatoes decreased by 29, 62, 76 and 83% in successive years compared to virus indexed "seed". It may seem that this vicious circle could readily be broken by simply not using infected material for propagation - and of course it could. However, many systemic pathogens do not cause obvious disease symptoms and these latent infections may pass unnoticed if yield declines only very gradually. Avoidance of infected propagation material requires tests for the presence of the pathogen. And if all the available material for propagation is infected, then steps must be taken to "clean up" the plant.

Before considering the role of plant tissue cultures in the control of diseases caused by systemic pathogens, it is pertinent to give a brief view of the losses that result. This will give an indication of the potential benefits that would accrue from the use of high health planting material.

LOSSES CAUSED BY SYSTEMIC PATHOGENS AND THEIR CONTROL

Estimating crop losses caused by plant diseases is a notoriously difficult field of study. The compilation by Cramer (1967) gives a unique picture of losses of world crops. An example of the information he gathered is given in Table 1. The figures he quotes must be considered as tentative because of the variable quality of the raw data (Smith *et al*, 1984) but they give an idea of the scale of losses.

TABLE 1

Pre-harvest losses of citrus (in 1000 tonnes)

	Actual production	Potential production	Total loss(%)	% loss caused by: insects	disease*	weeds
N. & C. America	8,470	10,603	20	7	9	4
S. America	4,210	5,910	29	10	15	4
Europe	3,996	4,701	15	7	5	3
Africa	2,273	2,990	24	10	10	4
Asia	5,166	6,378	19	8	7	4
Oceania	280	355	21	8	9	4
	24,395	30,937	21	8	9	4

*Principally virus disease and *Phytophthora citrophthora*

In some instances much better data is available. In potato, Bawden and Kassanis (1965) demonstrated a 10% yield increase in the cultivar King Edward freed from PVM infection. Similarly, Harper *et al* (1975) showed that yield losses of up to 60% occurred in field plots planted with potato seed that was infected with potato leaf roll virus. Work such as this has led to the development of the U.K. scheme for production of potato seed via virus tested stem cuttings.

Tree crops can also be seriously affected by systemic pathogens and in view of the longevity of the crop plant and capital expenditure to establish a plantation, healthy planting material is particularly desirable. Table 1 shows the loss caused by disease in the citrus crop worldwide. In apple, appreciation of the effects of virus diseases led to the development of the EMLA scheme in the U.K. to provide healthy rootstocks and scion budwood (Campbell *et al*, 1977).

In the tropics, vegetatively propagated crops like bananas, cassava, taro, cacao and sugar cane all suffer debilitating virus diseases. Recently, commercially produced virus indexed sugar cane has been made available in the U.S.A. And a recent report from the International Institute for Tropical Agriculture described the basis of a scheme for elimination of cassava mosaic from planting stock.

In ornamental horticulture many crops are vegetatively propagated and many have become loaded with systemic pathogens. For example, Logan and Zettler (1985) reported that gladioli "appear to be ubiquitously infected with viral and microbial pathogens". A huge taxonomic range of plants is grown for ornamental purposes and there are many systemic pathogens affecting them. Koenig (1985) reported that more than 80 new

virus diseases of ornamentals were reported in the period 1980-1985. Most of these reports were academic studies of the viral pathogens and no estimates of crop losses were made.

Chrysanthemum is an important glasshouse flower crop that can be programmed for all-year-round production. Horst et al (1977) have reported on the reduction of productivity of cut flower chrysanthemums due to two viruses and two viroids. Their results are summarized in Table 2 and show clearly the effect of the pathogens which result in reduced quality and profitability of the crop.

TABLE 2

Total percentage reduction in fresh weight, stem length, and flower diameter of 10 chrysanthemum cultivars due to viral and viroid infections during three growing seasons (%)

	Stunt viroid	Chlorotic mottle viroid	Aspermy virus	Mosaic virus
Fresh weight	29	10	18	17
Stem length*	15	5	11	10
Flower diameter*	9	1	5	4

*Stem length and flower diameter of only standard type cultivars

The evidence of crop losses shows that there is considerable opportunity for the production of disease indexed planting material. It can further be seen that the motivation to establish clean stock schemes comes as a result of a proper appreciation of crop losses.

In most instances such schemes will involve "cleaning up" of infected stock. This need not entail tissue culture techniques but as will now be discussed, there are great advantages to be gained from proper integration of "clean up", indexing and propagation when all these are carried out using tissue cultures.

PRODUCTION OF DISEASE INDEXED PLANTS USING TISSUE CULTURES

The term "disease indexed" is used to describe plant material that has undergone tests for freedom from particular systemic pathogens. These tests carry a high degree of statistical probability of certainty but there remains the possibility that indexed material contains an undetectably low level of infection of say a virus or viroid. Thus the term "disease-free" is not used (Constantine, 1985).

Disease indexing is an umbrella term for what is actually a three stage process of pathogen screening, clean up of the infected plant, and then pathogen indexing.

Pathogen screening

This process is aimed at specific systemic pathogens of demonstrated importance not at pathogens in general. The screening process involves an assessment of symptoms, if any, coupled with diagnostic tests matched to the type of pathogen under consideration. For example, the technique of inclusion body staining can be used to help confirm the presence of certain viruses. If the presence of the pathogen is confirmed then the clean up procedure is begun.

Clean up of the infected plants

The tissue culture technique employed here is called meristem tip culture. It exploits the fact that the region of the plant closest to the shoot meristem is generally free of any systemic contaminant. A tiny portion of the shoot tip comprising the apical meristem and two to four leaf primordia and measuring less than 0.5 mm is excised and cultured in vitro. With the appropriate stimuli, this tiny meristem tip explant can be regenerated into a whole plant.

Heat treatment of virus infected plants prior to excision of the meristem tip has been found to be advantageous in many cases; virus replication is inhibited increasing the chances of recovery of clean plants.

Chemotherapy may also be of value. For example, when chrysanthemum meristem tips were treated with amantadine 10% of plants recovered were free of chrysanthemum stunt viroid but no viroid-free plants were recovered from control cultures (Horst & Cohen, 1980). Antibiotics have also been used to recover bacteria-free philodendron plants.

Pathogen indexing

Indexing involves a programmed series of highly specific and sensitive tests for the pathogen(s) involved and is the key to the whole process.

The principal techniques used by Twyford Plant Laboratories to detect viruses and viroids are respectively enzyme linked immunosorbent assay (ELISA) and polyacrylamide gel electrophoresis (PAGE). Both techniques represent a considerable improvement over the use of indicator plants in terms of sensitivity and efficiency. For bacteria and fungi, selective nutrient media are used although modified ELISA procedures can be used for some bacteria. Research work at Twyfords is aimed at refining these tests to make them more sensitive and increase the vigour of the indexing process.

DISEASE INDEXING AND MICROPROPAGATION

Disease indexing should be viewed as only a part of a properly constituted strategy for control of systemic pathogens in vegetatively propagated plants. Eventual re-infection of indexed stock is possible so the ability to replace diseased stock rapidly or provide a steady supply of indexed material is important.

The tissue culture technique of micropropagation is of great value in propagating disease indexed plants. Once in culture, an indexed plant is completely protected from re-infection so that thousands of uniform indexed plants can be produced quickly and economically. Some plants cannot be micropropagated on a large scale because of somaclonal variation although tissue culture can be used in the clean up procedure. Giles and Worfolk (1985) have described a scheme developed by Twyfords for the

production of disease indexed chrysanthemum cuttings. The scheme combines tissue culture clean up and sanitary production of indexed cuttings in a vector screened glasshouse.

By programmes such as this and amelioration of conditions favourable to re-infection by the pathogen, a coherent strategy for control of disease can be developed.

While micropropagation can provide a programmed supply of healthy material to start each crop, husbandry techniques must be developed to prevent early re-infection.

COMMERCIAL CONSIDERATIONS

The role of tissue culture in producing disease indexed material is becoming ever more important although it is currently used only for a relatively small range of crops. Part of the reason for this is that disease indexing is a difficult service to market because there is still a rather poor appreciation of the effects of systemic pathogens. Many viruses cause no distinct disease symptoms and in the absence of good research studies demonstrating crop losses, it is difficult to establish the economic benefits to the commercial grower. Disease indexed material is, properly, more expensive than non-indexed.

This situation is compounded by a persistent fallacy that all micropropagated plants are inherently "clean" and free from disease. This is simply not true. Superficial contaminants are removed as part of the tissue culture process but systemic contaminants can be propagated along with the plants in culture. There is an important educational job to be done and it is expected that this will become easier as concrete results, such as those obtained by using indexed chrysanthemum cuttings, are achieved in commercial practice (Giles & Worfolk, 1985).

CONCLUSION

Tissue culture techniques offer a unique opportunity to produce and propagate disease indexed plant material. While the benefits of using such material are poorly appreciated, there are commercial opportunities for the production of healthy planting stock which is vital to ensure maximum expression of the benefits accruing through plant breeding programmes.

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Certification

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CLEAN STOCK SCHEMES: WITH EXAMPLES FROM SCOTLAND

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ABSTRACT

The development of clean stock (or certification) schemes Our understanding of diseases and the opportunities these new insights provide for the development of clean stock (or certification) schemes is illustrated by reference to the Potato, Narcissus and Soft Fruit schemes operated in Scotland by the Department of Agriculture and Fisheries for Scotland (DAFS). Additional factors, such as provision of phytosanitary certificates for exports and the relationship between technical capability and costs are discussed. Problems of cultivar supply and demand by commerce are contrasted with overall objectives for disease control.

INTRODUCTION

Whilst in the latter part of the nineteenth Century the benefit of obtaining new potato seed from Scotland or Ireland had been recognised in areas where "degeneration" occurred, it was only as our understanding of diseases and their biology began to emerge that it became possible to put clean stock or certification schemes on a more scientific basis. Many of these schemes are directed towards vegetatively propagated crop plants where the accumulation of infection has an increasingly deleterious affect on the productivity of the plants or their progeny. Conversely, if stocks free of disease can be generated and distributed then there is increased productivity and an economic gain. Ebbels (1979) has provided a historical view of clean stock schemes for England and Wales and whilst there is no comparable review for Scotland such schemes there have developed in parallel and sometimes led the way. The schemes for potato, strawberry, rubus and narcissus, operated by DAFS, have adapted new techniques for disease detection and control as they became available. By reference to our experience over the years in developing these schemes certain principles and problems can be illustrated.

POTATO SCHEME

Historically, today's potato certification schemes are a direct result of concern about the spread of wart disease in the early years of this century. Whilst legislative controls restricting planting of potatoes on contaminated sites (scheduling) helped, the discovery by Gough (1908) that some cultivars exhibited resistance (immunity) was the real key to control. Cultivars grown in contaminated soil could be classified immune or non immune but there were over 2000 potato variety names in use at that time, many of which were synomyns for the same cultivars. With further spread of the disease during the 1914-18 war the demand for wart immune cultivars led to the setting up of a Scottish Plant Registration Station in 1918 with the objective of developing a system to give assured

supplies of cultivars with a high level of uniformity (purity). Key personalities such as Dr Soloman and Dr McIntosh made possible the creation of order out of chaos by providing the basic systems for variety identification and inspection of crops which then received a certificate for purity if they reached the specified standard. Growers could then buy with assurance knowing the wart disease immune character of a stock. The effectiveness of the availability of pure material of known reaction to wart disease on the reduction in the number of new field infections is shown for Scotland by the reduction in field outbreaks from 206 in the decade 1917-1926 to only one in the period 1967-1976 (DAFS Records).

Testing new cultivars for wart disease reaction has changed from field to glasshouse/laboratory testing (Noble & Glynne 1970). More recently the European Plant Protection Organisation has specified criteria for establishing resistance in new cultivars (Anon., 1981) with the aim of improved confidence in the tests and in the resulting interchange of information.

Progress on other problems such as plant virus diseases were much less spectacular, as reliance had to be placed on such simple (to us) techniques as roguing and assessment of infection by counts during inspection. This progress was based upon the experience that the removal of infected plants could outpace the occurrence of new infections by viruses such as potato leaf roll virus and potato virus Y under climatic conditions where their spread was slow. Equally these conditions allow the multiplication of clones selected for freedom from such well expressed diseases. A progressive tightening of field inspection standards has ensured that infection levels in the progeny were such that buyers of seed could expect low levels of infection with the severe viruses. This is the measure of effectiveness of potato certification used by the EEC. Each Member State has to submit control samples for assessment, at a common centre, by a panel of experts from all Member States. Compliance with the standards set, which allow for disease development post inspection, is usually successfully achieved for the severe viruses in samples from the UK. Prediction of the extent of infection in progeny stock for a disease such as blackleg (Erwinia carotovora var, atroseptica) assessed at field inspection is less reliable, as disease development and extent of tuber infection is dependent on many factors. Similarly where a 'nil tolerance' standard is imposed, as for the veinal necrosis strain of PVY, absolute compliance cannot be assured by inspection of samples. Buyers should be aware of these limitations of field inspections.

Real progress was only possible when virologists devised both improved methods of testing and the ability to free clonal material from viruses by apical meristem culture. Virus testing in relation to generation of clean stocks has been extensively used for many vegetatively reproduced crop plants but it is perhaps taken for granted that sustaining the availability of such virus free material, once achieved, affects the pattern of management. Families of each cultivar have to be maintained and propagated in a way so as to minimise the risks of re-infection.

For potatoes such a system was first introduced in Scotland in 1950 (Foister 1961) with each family being tested for Potato Virus X and Potato Virus S according to a schedule based on its age. It is because these

systems of management must be in the hands of experts that responsibility for the generation of virus tested stocks has been done directly by DAFS since the early 1950s. The material is then released to growers willing to take the extra care needed for further multiplication. Sufficient material can be generated in four to six seasons from a single mother plant of any variety to supply the whole industry in Scotland. (Hardie, 1970, a & b).

Almost unwittingly a new idea emerged from this type of management system which enabled other disease problems to be tackled. That was the concept of displacement or flushing through with the healthy stocks displacing the older stocks with more disease. Rates of re-infection were outpaced in most years and so an overall improvement was possible both in the general level of virus infection and the number of crops passing inspection. The system was not, however, free from setbacks due to environmental factors favouring virus spread. It also induced some complacency with the older technique for maintaining health by roguing. A noteable example of this was the early 1970s when a high level of aphid activity increased potato lef roll infection rapidly leading to many crop rejections and the occurrence of the disease in a high proportion of all stocks (Howell, 1978). For the first time advice was given to use insecticides to avoid aphids transmitting PLRV early in the season. The advent of a chain of aphid suction traps plus research on overwintering of aphid vector species also allowed improved advice to farmers in years when early development of the aphid vector population was expected so that they could decide when the use of insecticide granules or subsequent spraying for aphid control is most beneficial (Turl 1980).

Whilst these developments for control of virus disease were in progress, the fungal and bacterial diseases affecting seed potato tubers were causing considerable concern, especially potato blackleg caused by Erwinia carotovora var. atroseptica, gangrene, Phoma exigua var. foveata and skin spot, (Polyscytalum pustulans. These problems became acute in the mid 1960s with many complaints about the amount of these diseases in seed potato stocks. Research had indicated that each of these diseases arose, primarily, from infected tubers and that the infection cycle starts from planting such tubers. If stem cuttings are taken before infection reaches them then the disease cycle can be broken and tubers free from these diseases produced. This was demonstrated for skin spot by Hide et al (1969). In 1967 DAFS initiated a pilot programme using this technique and, this being successfull, embarked on a full scale programme to replace all commercial material with that derived from stem cuttings. This system is described in detail by Hardie (1970 a & b). Other significant features of the programme were the use of isolated field sites for the initial multiplication to minimise re-infection and the use of laboratory test techniques to check on freedom from infection of the cuttings. Despite the early encouraging results a limited amount of infection was found at the new site in 1971 (Graham 1971) a year when blackleg was extensively found in many stocks. Investigation of this re-infection suggested that flies (Leptocera sp.) may spread infection and since then it has become apparent that the blackleg organism can also be carried as a rain induced aerosal (Graham 1976). Analysis of observations from crop inspections over the years has shown an improvement in the seed area meeting the standard for blackleg (0.25% or less) but also that the older the stock the poorer the

chances of the stock being free of the disease (Anon. 1985). This has led to a tightening of the concept of generation control with the reduction in the number of years stocks may remain in the Super Elite grade (formally Foundation Seed grade) from 3 to 4 years. In addition the change over from stem cuttings to micropropagation (Jeffries, this volume) has allowed a reduction of a year in the VTSC grade production period. The scheme requirements for burning down within 21d of the final inspection and the recommendation that tubers are lifted as soon as possible are husbandry measures aimed at blackleg control rather than aphid-borne viruses.

Control of the fungal diseases, gangrene and skin spot, has also encountered problems but again progress has been made especially where fumigation using 2-aminobutane, or spraying with thiabendazole is used after harvest to support the generation control system. More recently (Carnegie et al 1984) obtained good control of these diseases by combining these two chemicals as a low volume tuber spray treatment at harvest. Further progress is dependent upon reducing re-infection both in stores (Carnegie et al, 1978) and in the field. Better management of stores could limit dust generated by handling operations carrying these organisms from contaminated stocks to the more recently derived and cleaner stocks. Growers are reluctant to spend more on improved storage or handling as the premium for high quality seed does not reflect the increased costs.

The adoption of micropropagation requires even stricter testing for freedom from all diseases, which could be as readily multiplied as the plants. This made possible by recent improvements in test methods for viruses such as the ELISA test. Perhaps even more significant in this respect was the development of an antiserum for potato leaf roll virus, and for which no satisfactory test existed, as recently as 1980. The skills of the bio-chemist in finding methods for the extraction and purification viruses like PLRV are thus directly linked to the progress possible in ensuring clean stocks of propagating material.

NARCISSUS

A scheme for the certification of narcissus has operated in Scotland since 1969. Although there had been earlier attempts to start a bulb multiplication industry in Scotland, to take advantage of the environmental conditions that reduce degeneration due to virus diseases, it was not until the late 1960s that a group of farmers in the Angus /Kincardineshire area realised the potential of the crop and the ease of adapting potato machinery to harvest the crop that significant areas were planted. One of the aims of this group was developing exports so there was an immediate need to ensure that health standards were sufficient to comply with the requirements of the importing countries. Most countries require a growing season inspection for health followed by a pre-export dry bulb inspection and of course freedom from potato cyst nematode (PCN). Initially health was ensured by selection and roguing out virus infected plants and due to the low rates of re-infection and the establishment of rotational practice quality has been improved and sustained. Exports currently account for approximately half the output at 600t; divided between Sweden, Denmark, West Germany and Holland with a value around £0.2m.

The link between clean stock schemes and health requirements set down by importing countries in their phytosanitary regulations has gained in importance as bodies such as the European and Mediterranean Plant Protection Organisation (EPPO) increasingly recommend the use of derivation from clean stock, generation control, crop inspections and separation or isolation from possible sources of infection in their Standard Quarantine Requirements (SQRs) (Anon. 1982). Many of these practices have been incorporated in the EEC Plant Health Directives (Anon., 1977 and 1980) which obliges each Member State to adopt them and they then become a legal obligation in the movement of planting material.

The basis for the issue of phytosanitary certificates derives from the International Plant Protection Convention which is aimed at preventing the dissemination across national boundaries of pests and diseases of plants and plant products. Each country is required to have an official plant protection organisation and in the United Kingdom this task falls to the relevant Agricultural Department who are required to ensure that each consignment meets the requirements of the importing country. Where a certification scheme is also used as the basis for phytosanitary inspections the inspector has a dual role, as the information gained is used later to support an application for such a certificate. The departments also needs to have records on origin, virus testing, generation control and the status of other diseases in the area of production.

The narcissus crop also illustrates another dilemma for those responsible for clean stock schemes; the balancing of the availability of improved techniques with economic necessity. Mowat et al. (this volume) have described the development and current status of virus tested narcissus in Scotland and the release in 1985 of some 15-20t of Foundation Stock bulbs to the Scottish Nuclear Stock Association (Flower Bulbs) Ltd. This is the culmination of a joint programme begun in 1972 with the objective stated in their paper. The indications that re-infection with both aphid-borne and soil-borne viruses under the systems adopted are minimal is encouraging and there is now material available for commercial evaluation of the benefits that freedom from these viruses may produce. However, under current Government policy all clean stock schemes must be costed and economically viable. Whilst the inspection aspects of this Foundation Scheme meet these criteria, most of the supporting testing work done by DAFS has to date been done on the basis that the scheme was experimental. The selection of sites for the third (field) stage of the propagation programme involves the taking soil samples both for examination for the nematode vectors Longiderus elongatus and trichodorids and for bait testing for presence of the soil-borne viruses, tomato black ring, raspberry ringspot and tobacco rattle. Several samples are taken from each site and several sites have had to be tested to ensure finding at least one suitable for use after rejecting those with soils containing virus infected weed seeds and thereafter choosing the site with the lowest nematode counts prior to pre-planting fumigation. This selection process is expensive, both in the use of staff and facilities. It is, therefore, important that re-infection rates under less ideal conditions be assessed so that such costs can be balanced against benefits measured in terms of any improved performance of VT stocks and enhanced value. The certification scheme can then be designed to limit specialised testing

processes to those specific and critical stages most likely to sustain adequate control yet allowing the costs to be minimised to achieve costs growers find attractive. Similarly any testing for virus infection using the methods described by Mowat et al. (this volume) at selected stages in the multiplication system must also meet and justify these costs and the same criteria apply.

STRAWBERRY & RASPBERRY

The strawberry and raspberry certification schemes operated in the UK are also based on derivation from health stock. When a number of strawberry and raspberry cultivars became available as virus tested stocks in the early 1950s it again became apparent that some system of management was necessary to ensure that these stocks could be sustained. This lead to the setting up of the first Nuclear Stock Association to regulate the production and distribution of such clean stock. This gave a great impetus to the use of clean stocks by commercial growers but there are now difficulties with this system related to the introduction of many new cultivars. The Research Institutes and the National Seed Development Organisation, in which Plant Breeders Rights for all State bred varieties are vested, are primarily interested in the State bred cultivars, leaving a grey area for other new cultivars introduced to commerce. Particularly with strawberry where there have been many recent introductions of new cultivars from Europe and the USA the previously well ordered system has not been able to operate. MAFF have introduced the "Health Only" scheme for such cultivars. Whilst our import requirements specify certain standards in respect of strawberry viruses and we ask for freedom from strawberry red core disease (Phytophthora fragariae) (and here we have the reverse process to our own certification of exports), only when a variety has secured a commercial niche is there sufficient incentive for a virus tested stock to be produced which can then enter the "standard" scheme at "Elite" level. Thus for a period different standards can apply which could undermine the overall health situation. With a succession of new imported cultivars this weakness is perpetuated.

About 6 years ago it became increasingly clear to us in Scotland that, with respect to strawberry red core disease, the system of two field inspections was not always successfully in picking out stocks which became infected with this disease. Whilst the scheme required land used for strawberry certification to have no known history of this disease, in practice this proved insufficient because of the ability of this organism to 'migrate' in free water from an infected site to lower land. The development of a method for testing for this disease using easily infected bait plants (Duncan 1980) which permitted the testing of root tip material, allowed the introduction of an autumn root tip test into our scheme. Initially many crops of runners were rejected, but recent seasons have seen only a few rejections. We believe we are now able to offer an improved assurance, to buyers, on freedom from this disease (Howell & Rankin 1984). Our system of autumn planting and spring harvesting allows time for this test to be carried out in the late autumn prior to sale. We appreciate this is not so feasible with autumn planting and autumn harvesting as practiced in England, nevertheless, we are actively considering altering our existing Sale of Strawberry and Blackcurrants (Scotland) Order to require all stocks being offered for sale to have come from root tip tested

stocks. Growers have increasingly sought out land with no record of this disease or strawberry production to avoid the problem, they therefore need this improved assurance that the stocks they plant do not jeopardise their investment by carrying infection.

Our programme has been assisted by two other developments 1. the introduction of micropropagation of the virus tested material received from East Malling by the East of Scotland Agricultural College and 2. the same organisation offering growers an advisory soil test for the disease using the Duncan bait plant technique. The first of these has allowed a whole generation step to be saved in the multiplication process at the same time avoiding exposure to infection by red core. The second has eliminated at least those sites with infection within the limits of sampling error: notoriously difficult when checking for non randomly distributed soil organisms. At the moment this scheme only applies to a limited range of cultivars partly for the reasons described above and partly because the Scottish Nuclear Stock Association is primarily interested in meeting the predictable needs of commercial growers, but not the broader market covering new cultivars for the amateur market. Again this tends to undermine the improvements such technical innovation has made possible and one can give no assurance that in the longer term there will not be a deterioration in the red core situation.

A similar, but perhaps more urgent dilemma, exists with the Raspberry Certification Scheme. Again only some cultivars are available as virus tested root cuttings to feed into the certification scheme, although the scheme in Scotland does include Tayberry and in England, Loganberry. Currently several newer continental cultivars are being promoted. The concern is the recent discovery of a resistance breaking strain of raspberry bushy dwarf virus (RBDV) (Barbera et al., 1984) a disease which occurs in the UK but to which most UK bred cultivars are resistant. The resistance breaking strain could adversely affect all these resistant cultivars. Because the virus is transmitted in pollen from infected plants, spread is much more likely than for a soil-borne pathogen. Surveys to date indicate that this resistant breaking RBDV is limited to some Loganberry cultivars. As the raspberry industry in Scotland is worth some £7m annually, much is at stake and we hope to see early progress in ensuring that all Rubus cultivars are derived and propagated from virus tested stocks. Whether this can be achieved before this resistance breaking form of RBDV establishes itself is dependent upon a willingness of those concerned with the industry to use only tested stock.

CONCLUSION

Over the decades developments in understanding of diseases and their detection have either permitted the establishment of clean stock schemes or contributed to their development. Sometimes the promise of new methods has been frustrated by a hitherto unknown aspect of disease etiology. Nowhere is this more true than with pathogens causing latent infections. Other pressures such as the need to meet increasingly specific criteria for the issue of phytosanitary certificates for export combine with these new ideas to make clean stock schemes more complex and costly. A balance has to be struck between what can be achieved technically and what industry is prepared to pay or support. There is however a need for busy growers and farmers to understand that clean stock schemes offer economical means of

controlling some diseases for which there are no other economic control measures available. This is especially true for soil contaminating pathogens which limit a growers freedom of choice.

Environmentally our climate offers many opportunities for the development of these schemes because re-infection rates are slow for many virus diseases. Also we often have the advantage that some diseases and pests or particular pathotypes of them do not exist here. Providing we take precautions to ensure they are not introduced with imports then this assists when freedom from these diseases is specified in the import requirements of other countries. Given the improved methods of propagation and disease detection now available, opportunities exist to further extend our utilisation of clean stock schemes to ensure the health and productivity of vegetatively propagated plants.

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CERTIFICATION AND ITS VALUE TO THE FRUIT GROWER

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ABSTRACT

During the past 20 years the development of pathogen-tested clones of all the major tree fruit cultivars has improved the growth and production of young trees while fruit quality and yields have been increased. The production of such clones and their maintenance and multiplication under the Plant Health Propagation Scheme is vital to the UK tree fruit industry. The close co-operation which exists in the UK between government, research station and grower is essential to the success of the certification scheme and must continue.

INTRODUCTION

The fruit growing industry has experienced many changes over the last 20 years. Before this date, virus diseases were prevalent in almost all the commercial tree fruit cultivars. Prior to 1965 efforts had already been made to multiply sources which were free from the known viruses, and also from rubbery wood and chat fruit. Subsequent research showed that almost all of these commercially accepted clones still contained the so-called "latent" viruses. Developments in research showed these viruses could be eliminated from fruit cultivars by the application of a heat treatment technique followed by rigorous testing of the treated material on sensitive indicator plants to ensure complete freedom from all known viruses. Only healthy clones were considered for issue.

It is fortunate that the research stations recognised the immense potential value of these selected clones and realised they could not allow haphazard introduction. East Malling and Long Ashton Research Stations formulated a scheme which became known as the "EMLA" scheme, which was designed to assure the authenticity and health status of all the cultivars issued. This prefix is now universally regarded as a hallmark of excellance.

Fruit growers and commercial nurserymen were quick to observe the improvement in growth and cropping potential of the healthy material and also soon realised that this valuable asset should be carefully handled and monitored if the health status was to be maintained. Therefore in 1966 a small group of nurserymen, under the guidance of the late Mr C.P. Norbury, formed the Nuclear Stock Association (Tree Fruits) Ltd. Its members were committed to maintaining and multyplying the healthy clones under the stringent conditions of the Special Stock certification scheme set up by the Ministry of Agriculture, Fisheries and Food (MAFF) for this purpose.

More recently the various certification schemes for individual fruit species have been amalgamated into the Ministry's Plant Health Propagation Scheme (PHPS). It is important to realise that this is a voluntary scheme, but never-the-less is extremely well supported by the fruit growing industry, and it must be noted that it is largely financed by the nurserymen who enter their plants for certification.

ORGANISATION FOR CERTIFICATION

The general standards of the PHPS are upheld by the close collaboration of the MAFF Plant Health Division and Agricultural Development and Advisery Service, including the Harpenden Laboratory, with the Agricultural and Food Research Council and the Nuclear Stock Associations. The excellent relationship and goodwill which exists between these organisations is widely recognised at home and abroad.

The Nuclear Stock Association (Tree Fruits) Ltd, holds regular board meetings to which representatives of all the organisations concerned have standing invitations. Their counsel and advice is greatly appreciated in the discussions of growers' problems or concerns and often immediate solutions are arrived at. This democratic approach and sharing of ideas means the industry readily cooperates with the administrators and difficult decisions are more easily accepted. Many people regard our certification schemes equal to, or better than other plant health schems elsewhere in the world and some find it difficult to understand how such close collaboration can be achieved.

The MAFF Plant Health Division administers the scheme and issues certificates on the basis of reports from the inspecting officers (the ADAS Horticultural Advisers). The stock is grown under the strict conditions laid down under the PHPS which are designed to maintain the health status and quality of the high grade propagating material.

BENEFITS OF CERTIFIED STOCK

Fruit tree nurserymen know that the maintenance of healthy propagation material is fundamental to the well being and long term future of the UK Fruit industry. They also realise that healthy plants grow better in their nurseries; bud take, survival rate, quality, and grade out are greatly improved. Fruit growers are also well aware of the dramatic economic benefits of using healthy certified planting material. Increase in total crop from healthy trees has been well authenticated and such stock is therefore a sound investment and provides the best foundation for a prosperous industry, the value of which now stand at approximately £222 million if the soft fruit contribution is also included.

FIELD INSPECTION

The field inspection of the growing stock, to check health, vigour and trueness to type is the bedrock on which the reputation of certified stock has been built. The annual inspections are carried out by experienced ADAS officers who have built up their expertise in this work. Most growers welcome the visit of these inspectors: they know that the crops will be completely walked and examined by experts, and the growers know that it would be impossible for them to carry out this task themselves. The inspectors, whilst having a firm approach, also show a great deal of tact and diplomacy and are respected by most of the horticultural trade.

I believe there is a great value in using ADAS Horticultural Advisers for inspecting the growing crops, since their everyday contact with the industry allows them to provide on-going monitoring of commercial stock, so that disease outbreaks and spread can be minimised and inferior stocks weeded out.

THE FUTURE

Stock of doubtful origin and health would lead to a rapid decline in the productivity of the industry and would reduce our competitiveness and self sufficiency in this important sector of horticulture.

For the future it is of major importance that virus testing, and the removal of other pathogens of new cultivars and the recloning of other cultivars required by commercial growers should be an integral part of a fruit research station's activities.

Once healthy stocks have been produced, the maintenance of a number of mother plants of each cultivar by the research stations concerned is essential and adequate funding must be available for this work. The Nuclear Stock Associations should then be able to draw on this nucleus of material periodically for whichever commerical cultivars are required by the industry in order that they can be propagated under the terms of the MAFF Plant Health Propagation Scheme.

I believe it would be very difficult to determine accurately the real value of certified stock but government expenditure would seem to be extremely modest for such fundamental and major economic benefits to the fruit and hops industry. The industry has already shown that it is willing to pay its share, where it is considered necessary, and any economies which are to be made should not devalue the high international standing of the UK Plant Health Schemes.

CERTIFICATION OF VARIETIES AND VARIETIES OF CERTIFICATION

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ABSTRACT

Certification schemes developed during the past 70 years cover both vegetatively-propagated and seed-propagated crops and may be voluntary or statutory. According to grade and design, they give various degrees of assurance that the planting material is of the stated cultivar and contains less than stated amounts of disease, pests and botanical variants. Schemes vary in complexity from the simple, designed to control one disease or for very limited purposes, to the complex, which can facilitate control of many different diseases, assure trueness to cultivar, and enable the material certified to meet plant health regulations.

INTRODUCTION

Certification schemes have been developed and diversified considerably since their inception about 70 years ago (Ebbels, 1979a). Early schemes were designed primarily to ensure trueness to variety, although this may have been a major factor in disease control, as with immune cultivars of potatoes for control of wart disease. To-day, schemes for certification of true seed, such as those for cereals and field beans, are still mainly concerned with botanical purity: those for vegetatively-propagated crops place equal or more emphasis on plant health and disease control (Hollings, 1965).

TYPES OF SCHEMES

Early candidates for certification among vegetatively-propagated crops were those in which cultivar recognition is difficult and in which (perhaps as a consequence) cultivar nomenclature was confused. Potatoes (Shepard & Claflin, 1975), strawberries and black currants are examples. With the advances made by plant pathology (especially in plant virology) during the 1930s, the need to improve plant health was increasingly recognised. This resulted in the incorporation of health criteria into the Scottish seed potato scheme in 1932 and into the England and Wales scheme in 1940. Since then, health has been a major consideration in UK schemes for vegetatively propagated crops.

Simple schemes

The simplest type of scheme is that designed for a particular and limited purpose, such as the Scheme for the Inspection and Certification of hop gardens operated by the Ministry from 1943 to 1974. This was designed only to minimise the risks of transmitting progressive verticillium wilt of hops in the 'strap cuts' taken from fruiting plants which at that time were the normal propagating material. With the very wilt-sensitive cultivars then being grown, freedom of the hop plantation from symptoms of the disease thus gave reasonable assurance that material taken from it would be free of wilt. The development of wilt tolerant/ resistant cultivars and new methods of propagation by layering bines in special nurseries or by rooting cuttings under mist made this kind of

scheme obsolete for hops, for which a more sophisticated scheme designed for specialist propagating nurseries was developed. However, the difficulties of cultivar recognition precluded certification for botanical purity, although certificates are not given if there are any grounds for doubt on this point.

Development of scheme conditions

Diseases amenable to control by crop certification are those transmitted in or on the propagating material whose other means of spread can be controlled or avoided and which produce symptoms visible to an inspector. As they have developed, certification schemes have therefore increasingly incorporated strategies to minimise alternative means of spread (Ebbels, 1979b). Strategies to minimise spread by aerial transmission include isolation, control of vectors, vector avoidance, interference with vector transmission, removal of inoculum sources, deblossoming and post-harvest testing. Some of these also minimise secondary spread below ground, for which measures such as crop rotation, tests for soil infestation with virus vector nematodes and chemical soil treatment are also designed. Measures to minimise secondary spread by contact include testing and selection, pedigree control and hygiene during inspections and cultivations. Depending on the crop concerned, most of these measures and strategies feature in the main schemes operated in England and Wales for potatoes, berry fruit, tree fruit, hops and ornamentals.

Specialised schemes

In recent years special schemes have also been developed to cater for certain kinds of material or for particular methods of propagation. The MAFF schemes for micropropagated stock and for propagation of fruiting varieties of *Rubus* and *Ribes* by softwood cuttings are examples of the latter, while the strawberry Quarantine scheme and the Health grades operated for fruit plants are examples of the former. It is usually impossible visually to detect systemic infections carried through propagation by micropropagated plantlets, so in this case the scheme relies on inspection of the mother plants and a sample of the grown-on progeny, authenticity of pedigree, and checks on the propagation process. The Quarantine scheme for strawberries aims to cater for the initial propagation of new cultivars pending indexing, while the Health grades cater for material of cultivars which cannot be confidently verified but which satisfy basic health criteria.

STATUTORY CONTROL

Table 1 shows the various schemes currently operated in England and Wales for vegetatively propagated crops. At present only the seed potato classification scheme is statutory (implementing the EC Directive on the Marketing of Seed Potatoes) while all the schemes comprising the Plant Health Propagation Scheme are voluntary. Several EC Member States now have statutory schemes (or schemes which are effectively compulsory) for crops other than potatoes. Interest in compulsory schemes covering whole sectors of horticultural material, such as hardy ornamental nursery stock, is increasing and if this is taken up by the EC Commission it will advance the time when more of our own schemes are supported by legislation.

TABLE 1

Certification schemes for vegetatively-propagated crops operated by the Ministry of Agriculture, Fisheries and Food in 1985

Statutory		Voluntary	
Seed Potato Classification Scheme		Plant Health Propagation Scheme	
	Grades		Grades
Basic Category:	VTSC Super Elite Elite, AA	Berry Fruits Strawberries	Foundation, Super Elite, Elite, A, Health, Quarantine
Certified category:	CC	*Ribes*, *Rubus* Cuttings:	Foundation, SS, A
		Ribes, *Rubus* Field grown:	Special Stock, A, A (Health)
		Tree Fruit Apples, Cherries, Pears, Plums, Quince (rootstocks)	Foundation, Elite, SS, SS (Health), A, A (Health)
		Hops	A-plus
		Ornamentals Carnations	Foundations, SS
		Narcissus, Iris	VTMS, Foundation
		Micropropagated stock	

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2.
Viruses

Chairman : R. T. Plumb
Session Organiser : W. P. Mowat

1986 BCPC MONO. No. 33 SYMPOSIUM ON HEALTHY PLANTING MATERIAL

VIRUS DETECTION AND IDENTIFICATION

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ABSTRACT

There is no universal strategy for plant virus detection but bio-assays based on knowledge of symptoms produced and the diversity of experimentally infectible hosts are broad spectrum aids to diagnosis. Electron microscopy of plant extracts is more rapid and frequently allows tentative identification that can be confirmed using criteria such as the type of the essential nucleic acid and the strategies by which information encoded in the nucleotide sequence is translated into proteins. A diverse array of tests exploiting antigen-antibody recognition are available to detect virus particles in large concentration and, when virus particles are few or localized, enzyme amplified immunoassays using chromogenic, fluorogenic, chemiluminescent or radioactive substrates are the detection system of choice. Viruses that exist within plants as nucleic acids can be detected with radioactive oligonucleotide probes having sequences complimentary to those of the agents being sought.

INTRODUCTION

Plant disease, and therefore its corollary health, is a somewhat variable quality. Harmful deviations from normal growth do not inevitably result from virus infection of a plant; symptom severity is modified by environmental factors (notably temperature), and reactions to viruses tend to be transient. Plant reactions also tend to be localized at least in part because of multiple meristems and somaclonal variation within an individual that results in a mosaic of cells which are not all equally responsive to or infectible by viruses to which they are exposed.

Local discontinuities in vascular communication also contribute to uneven virus distribution that is especially noticeable in deciduous trees which, in summer, have foliage varying widely in morphology, anatomy and physiological age (e.g. Cooper & Edwards 1981). Virus populations are by no means genetically fixed and are themselves a source of variability in pathology: reflecting differences in temperature requirements for replication, the dominant virus genotypes vary throughout the year. Thus, an assay system to be dependable has to accommodate numerous variables and is difficult to standardize even for one host/virus combination in a circumscribed climatic region. It has been calculated (see Matthews 1957) that 46,000 individual plants must be tested and found to be virus free (with a 99% reliability) given an infection incidence of <0.01%. The confidence placed on health testing therefore depends on the sensitivity of the virus detection system used and these vary with the properties of specific viruses. None of the current techniques is equally responsive to the unexpected products of infections involving different viruses that are commonplace in the field. In this paper, a few examples have been given to indicate the strengths and deficiencies of diagnostic procedures that range widely in sophistication, convenience and sensitivity.

IDENTIFICATION

What is a virus?

The concept of a virus has evolved considerably since c. 1898 when this group of parasites was first distinguished from bacteria on the basis of size. In essence, viruses are distinctive ribose or deoxyribose nucleic acids dependent upon living cells in which they replicate either alone or in concert with other viruses. Viral nucleic acid necessarily codes for at least one enzyme which provides for its own replication but, in many instances, also directs the synthesis of specific protein capable of coating the nucleic acid thereby forming a particle detectable in an electron microscope. Pathogenicity is not an inevitable attribute: viruses typically cause few noticeable effects in wild plants but crop improvement has resulted in novel virus-host combinations and the spread of viruses that were hitherto geographically localized with decidely harmful results.

Identification

Viruses are identified on the basis of several biophysical characters such as nucleic acid size, type and ultimately nucleotide sequence as well as particle shape and, where appropriate, protein composition (see Hamilton et al. 1981). In addition, biological characteristics such as the requirement for and relationship with vectors has taxonomic value and knowledge of all these properties has been used to assemble viruses into families or groups (Matthews 1983) thereby providing a useful means of predicting attributes of newly found viruses after partial characterization. Thus, it is often possible to infer biological properties likely to influence the rate and patterns of natural spread thereby helping to rationalize containment/eradication strategies that may need to be formulated urgently. Where appropriate, immunological procedures that exploit differences largely in the proteinaceous components of viruses can be used to rapidly group a newly identified agent with others characterized previously. However, in the absence of a suitable range of reference sera or in laboratories lacking facilities appropriate for physiochemical characterization, comparison of experimental host ranges/symptoms (e.g. Tobias et al. 1982) or recognition of protection against superinfection afforded by a known isolate against an unknown (Fulton 1978) has value in differentiating viruses within groups.

DETECTION

When the objective is large scale testing aimed at optimizing the productivity of an infectible crop by planting specific-pathogen-free stock (or the diminishment of potential sources of inoculum) virus detection relies on one or more of the following: bioassays exploiting viral pathogenicity, microscopy, serology or radiological/colourometric assays.

Biological detection

Symptomatology

Examination of tubers, bulbs, seeds and deciduous woody plants in winter is rarely helpful in detection or diagnosis but the presence of viruses can, in some instances, be inferred from the external appearance of growing plants by identifying the abnormal or substandard within plant populations. Visual assessments are inexact but can help to narrow the range of possibilities. For example, flower breaking in tulips could indicate infection with either of two potyviruses; tulip breaking or tulip chlorotic blotch (Mowat 1985). Similarly, when brown lines are visible in stored potato tubers it is possible to guess at the possible presence of, for example, tobacco rattle tobravirus or potato moptop tobamovirus. Visual inspection of growing crops and removal (rougeing) of suspicious or frankly

diseased plants is a long established complement to certification schemes that aim to maintain the purity and productivity (health) of clonal crops (Ebbels 1979) but more discriminating tests to detect infectious agents should be applied in parallel. No single test is fool proof but in this instance safeguards are particularly necessary because rougeing tends to favour plant genotypes (cultivars) lacking symptoms while infected by viruses capable of diminishing the yield or quality of neighbouring crops (e.g. Shepard & Claflin 1975).

Indicator hosts

Many viruses can be transferred mechanically to 'new' hosts that show symptoms consistently when infected and which can be used as a means of detection: some of these yield the large amounts of virus required for detailed characterization. Extensive lists of infectible species and descriptions of virus-associated symptoms have been assembled (e.g. Horvath 1983) and some are computer accessible (e.g. Virus Identification Data Exchange; Boswell & Gibbs 1983). This information is useful for other purposes as when separating components of naturally mixed infections but plant reactions can vary with the virus isolate and also the stock of indicator plant even when inbred (e.g. van der Want et al. 1975).

Vector transmission

Knowledge of natural methods of virus transmission between plants almost invariably has value in diagnosis and repays labour intensive investigation. There are viruses (e.g. cherry leaf roll, Cooper et al. 1984) for which the normal transmission route is vertical via pollen to seed and others (e.g. cryptic viruses) that have not been convincingly shown to be transmitted any other way. However, a diverse array of organisms naturally penetrate plant cells and thus have access to viruses that they may acquire and introduce into cells of other plants which they subsequently wound, usually while feeding. Insects, arachnids, nematodes and fungi have all been implicated as vectors for specific viruses (for a general account see Cooper & MacCallum 1984). Recognizing the sophistication of virus-vector interactions and the taxonomic, manipulative, statistical and cultural constraints on experimentation, natural vectors are not eagerly used for routine virus detection. Nevertheless, because vectors tend to seek out specific tissues such as phloem in which luteoviruses accumulate and from which manual transmission cannot be effected, aphids were until recently essential aids to the diagnosis of, for example, potato leaf roll virus or specific biotypes of barley yellow dwarf virus.

Graft transmission

Grafting, especially between tree roots is suspected to be an important means of natural virus transmission and the experimental grafting of buds, bark, leaves or stem pieces into seedling or clonal indicator plants is routinely used to infer the presence of viruses in tree fruit or berry crops or to distinguish pathotypes of a virus having many other properties in common e.g. resistance-breaking isolates of raspberry bushy dwarf virus (Barbara et al. 1984). Traditionally, the indexing of fruit trees has been done in the field and such tests are in a few instances the most successful and sensitive means of detection (e.g. Spiegel et al. 1984). However, because of cost in terms of land, labour and maintenance, fruit tree testing is now often done in the more reproducible environment of glasshouses free from extraneous insect transmitted viruses and where the time for symptom development can be diminished from years to weeks (Friedlund 1980). To obtain transmission it is not in all instances necessary to have a perfect graft union and transmission has been obtained between unrelated

taxa e.g. transmission of grapevine fanleaf virus from Vitis to Chenopodium amaranticolor Coste & Reyn. (Cadman et al. 1960). When the problem occurs, it can be circumvented by using polyphagous parasitic vines (Cuscuta spp.; dodder) to bridge between virus donors and recipients (Fulton, 1964). When the scion is abnormal and the stock initially healthy, the presence of transmissible pathogens capable of inciting disease in the stock can be distinguished from the effects attributable to insect toxaemia, nutriment imbalance or local cytological/ genetic change in plants. However, symptoms may not be virus specific (Smeets & Wassenaar 1956) and grafting bioassays do not allow viruses to be distinguished from bacteria or mollicutes (Hopkins 1977; Whitcomb & Tully 1979). Since these micro-organisms were first recognized in plants, progress has been made in providing means for their identification. Thus, in a few instances, they may be identified using physiological and morphological attributes in culture. Alternatively or additionally, the agents can be demonstrated in situ by DAPI (Seemuller 1976) or Dienes staining (Deeley et al. 1979), by nucleic acid hybridization (Boulton et al. 1984), by enzyme-linked immunosorbent assay (Clark et al. 1978) or, as originally, by electron microscopy (Doi et al. 1967).

Microscopy

Histological/cytological examination of plants has been used in virus diagnosis (see Christie & Edwardson 1977) but is unfashionable because it is time consuming and tests such as staining for callose to indicate the presence of potato leaf roll luteovirus in tubers (de Bokx 1967) are less reliable than alternative procedures. Electron microscopy is routine and especially useful when surveying crops such as Agaricus bisporus (e.g. Del Vecchio et al. 1978) from which virus transmission cannot easily be achieved. However, in the absence of such pathological information, ignorance continues about which of the morphologically distinguishable virus-like agents recognized causes significant disease (currently there are at least 6 virus-like agents in mushrooms). In higher plants or vectors the characteristic morphology of the particles of geminiviruses, rhabdoviruses and reoviruses permits unequivocal detection. Furthermore, the modal length of a virus population having filamentous or straight tubular particles, has taxonomic value (Brandes & Berks 1965). Some viruses with isometric particles can be tentatively identified to group level (e.g. bromoviruses being distinguishable from tombusviruses or ilarviruses) with less than ten minutes of sample preparation and examination time but require an experienced electron microscopist. Nevertheless, isometric particles can be confused with ribosomes in plants or galactogen in snails (Kassanis et al. 1984) and aggregates of P-proteins resemble filamentous virus particles (e.g. Atkinson & Cooper 1976) and thus interpretation is not always unequivocal. However, the objectivity of electron microscopy as a diagnostic tool has been considerably increased by the use of immunological reagents as in the decoration of virus particles with antibodies and the labelling of virus specific proteins in tissue sections with antibodies conjugated to colloidal gold or ferritin (e.g. Milne 1984).

Serology

Plant virologists have long taken advantage of the immunogenicity of virus particles as a criterion for identification and, where appropriate, routine detection (e.g. Ball 1974; van Regenmortel 1982). The propensity of antiviral antisera to combine with and precipitate viral antigens is used to facilitate identification and quantitation (especially of tubular/filamentous virus particles when reactions occur in liquids). Procedures in which one or both of the reactants (antigens and antibodies) migrate through a gel

matrix have become routine because of their technical simplicity, because they provide additional information on antigen heterogeneity in size (or when used with electrophoretic methods, surface charge; e.g. Kobayashi et al. 1984), and because they also detect immunoglobulins against extraneous plant proteins which frequently contaminate virus preparations used to produce antisera (see van Regenmortel 1963).

When either or both of the reactants are in short supply, antigen-antibody complexes can be observed with the aid of an electron microscope but numerous alternative amplification strategies are available. The simplest of these is the aggregation of virus-coated chloroplasts which results within minutes of adding antiviral sera to crude foliar extracts. However, although much used in the detection of potato viruses X, Y, A and S (van Slogteren, 1969) this adsorbtion-precipitation reaction is appropriate for only a few viruses that plants contain in large amounts and has to a large extent been superceded by the more generally applicable and sensitive flocculation procedures in which inert substances such as latex beads (Talley et al., 1980) or bacteria (Staphlococcus aureus; Chirkov et al. 1985) are coated with antivirus antibodies. Immunoglobulins also adsorb to plastics (without greatly impairing their avidity/affinity for antigens) and this fact has been crucial to the success of the most sensitive serological tools. Antigen-antibody recognition on solid surfaces was most conveniently quantified when radioactivity labelled antibodies were being used to assay antigens. However, despite the technical simplicity of radioimmune assays, the environmental hazard and the short shelf life of convenient radio-nucleides such as ^{125}I is contributing to a decline in their popularity. Their place as tools in virus detection is being occupied by a family of techniques that exploit amplification systems in which enzymes act on chromogenic, fluorogenic, chemiluminescent or radiolabelled substrates (Cooper & Edwards, 1986).

Enzyme amplified immunoassays

Enzyme-linked immunoassay (ELISA) have diverse biomedical/veterinary applications and numerous modifications in the technology has been described (e.g. Maggio 1980). Protocols applied to the detection of viruses in plant extracts have also been reviewed (e.g. Clark 1981; Bar-Joseph & Garnsey 1981; Clark & Bar-Joseph 1984). Consequently, only a brief overview is approriate here. For reasons of cost, horseradish peroxidase has been used in preference to alternatives (notably alkaline phosphatase) which are more easily conjugated to immunoglobulins despite the fact that many chromogenic substrates appropriate for peroxidase are suspected mutagens. Urease is an enzyme recently used in ELISA (Evans et al. 1983) and has the prospect of becoming routine because it catalyses the release of ammonia from the low cost substrate urea. The product of this reaction can be conveniently detected by a pH indicator such as bromocresol purple which unambiguously changes colour from yellow to purple. Alternative substrates yielding brightly coloured products facilitating visual assessment include histological dyes such as fast red TR salt or fast blue BBN salt for alkaline phosphatase and, with less permanent results, horseradish peroxidase (Bantarri & Goodwin 1984). Fluorogenic (e.g. Torrance & Jones 1982), chemiluminescent or radioactive substrates provide worthwhile additional signal amplification in ELISA but require expensive detection systems and seem more appropriate for research than routine purposes of virus detection.

Although numerous solid phases have been used in ELISA to support antigen-antibody complexes, moulded plastic plates facilitated automatic

measurement and recording of virus detection data and have become standard. Much of this equipment has been designed around the 96 x 300µl well microtitration system but Terasaki plates with 10µl wells allow more conservative use of reactants and are satisfactory when yes/no answers are required (Edwards et al. 1983). Substituted cellulose sheets having greater uniformity (Burrows et al. 1984) and avidity for immunoproteins than plastic (Palfree & Elliott 1982) have been used in several forms of ELISA (e.g. Shukla et al. 1983; Bode et al. 1984) and seem to offer potential benefits of convenience and enhanced sensitivity of detection.

ELISA systems, in which virus specific immunoglobulins are directly linked to enzymes, are specific but their sensitivity is adversely affected by the chemical process of enzyme conjugation that tend to polymerize the immunoglobulin thereby diminishing by steric hindrance their affinity for antigens. Biospecific bridges involving biotin-avidin or lectin-polysaccharides seem promising alternative strategies (Kendall et al. 1983). Indirect ELISA systems differ in the methods used to immobilize viral antigens or immune complexes to solid surfaces. In one of the simplest indirect systems, virus is adsorbed to plastic passively from plant sap and detected using virus specific immunoglobulin or rabbit serum (cell-free component of whole blood) that are assayed with, for example, anti species (e.g. anti-rabbit serum prepared in pigs) enzyme conjugates (e.g. Koenig, 1981). Staphylococcus aureus protein A binds to the Fc portion of immunoglobulin molecules and not the antigen binding Fab fragment, is commercially available and can be conjugated to a variety of enzymes as well as latex beads, fluorescein, gold and radionucleides (see Richman 1983). Edwards and Cooper (1985) used protein A in an indirect ELISA system to bind and orientate virus specific IgG when making comparisons between antigenically distinguishable isolates of cherry leaf roll virus, and others have used protein A enzyme conjugation (e.g. Barbara & Clark 1982). Protein A reacts poorly with, for example, avian antibodies which is an important negative feature recognizing the trend towards immunoglobulin production in hens eggs rather than the more traditional rabbits, mice or guinea pigs.

ELISA protocols have been modified to diminish the number of steps required in routine virus detection (e.g. Flegg & Clark 1979; van Vurede & Maat 1985; Stobbs & Barker 1983; Mowat 1985). Furthermore, ELISA systems have the potential for enhanced precision and sophistication when monoclonal antibodies elicited against distinct amino acid configurations (epitopes) on viral antigen become more widely available (Yolken & Leister 1984; Schonherr & Houwink 1984). Monoclonal antibodies potentially eliminate the variation inherent in whole animal systems of serum production (when produced from hybridomas directly but not when amplified in mouse ascitic fluid), should have maximal usable sensitivity and are currently marketed for routine virus testing of potato stocks. The limitations in absolute sensitivity of different ELISA systems are attributable to the reversible nature of antigen-antibody reactions, inequalities of virus distribution in plants and unpredictable heterogeneity in the viruses themselves. Furthermore, plant species differ in the amounts to which their constituents interfere with amplifying systems and may generate false positive data (e.g. Torrance 1980; Mink et al. 1984). However, ELISA is not constrained by inadequate signal generation/detection. Fluorogenic substrates in solution are capable of revealing about 5000 molecules of β-galacosidase and when radioactive

substrates are used, ELISA reportedly detect as little as 600 molecules of cholera toxin (Harris et al. 1979). Minimal limits for virus detection or antibodies are greater by several orders of magnitude (Labrousse et al. 1982). Nevertheless, the various ELISA systems have enabled routine detection of tree viruses with filamentous particles (e.g. citrus tristeza; Bar-Joseph & Garnsey 1981) and phloem restricted luteoviruses (e.g. potato leaf roll; Tamada & Harrison, 1980; beet western yellows; Hewings & D'Arcy 1984) on a scale and with a reliability and convenience greater than similarly sensitive alternative procedures such as immunosorbent electron microscopy (Kojima et al. 1978; Roberts et al. 1980).

Nucleic acid hybridisation

When a virus is prone to antigenic variation or occurs without coat protein (e.g. tobraviruses; Harrison & Robinson 1978), immunological detection systems are unsuitable. Furthermore the coat proteins of viruses are thought to represent only a small part of total coding capacity of viral genomes and detection systems reflecting a greater proportion of the nucleotide sequences should be more discriminating. The complete sequence of nucleotides in the genomes of a few viruses have been determined and, despite the cost and labour intensiveness of acquiring sequence data, this information is being sought to facilitate construction of detection systems that recognize replication features common to a broad spectrum of viruses. Theoretically, radiolabelled single-stranded nucleic acids are capable of recognizing and discriminating between complementary sequences differing in only a few nucleotides. However, this precision may be unusable when assaying natural virus populations: copy error rates in RNA replication have been estimated as one base replacement in 10,000 (van Vloting-Doting et al. 1985).

Indirect methods for comparing viral nucleic acids are routine and have been used to detect viroids (Owen & Diener 1984) or tobraviruses in narcissus (Harrison et al. 1983). When the viral genome is single-stranded RNA, the assays depend upon the action of an enzyme (RNA directed DNA polymerase) catalysing the production of representative single-stranded molecules (cDNA) complementary in sequence but containing some radiolabelled nucleotides. The same technology is appropriate when incorporating incomplete complementary nucleotide sequences into extra-chromosomal components of bacteria that thereafter produce large amounts of a uniform probe molecule. This process has the additional benefit of minimizing the need to cultivate potentially exotic pathogens in an infectious form thereby diminishing the phytopathological risks inherent in anticipatory phyto-sanitary research. Viral DNA or double-stranded RNA can be radiolabelled and propagated analogously and used to produce probe molecules capable of detecting viruses in extracts of plants or insects dotted onto nitro-cellulose sheets (e.g. Maule et al. 1983). This is a more convenient procedure for routine diagnosis than the more discriminating but also technically exacting nucleic acid hybridization in solution (e.g. Palukaitis & Symons 1980).

Nucleic acid hybridization can detect as little as 2.5 pg (per 100µl) of purified RNA (e.g. Sela et al. 1983) but is not in all instances more sensitive than biological assays or ELISA (detecting 0.2-2.0ng/100µl): the relative sensitivities of the procedures vary with the protein:nucleic acid ratio of the virus particles being sought and their specific infectivity. The short shelf life and health hazard of radionucleides limits their use in

routine plant virus detection and alternative nucleic acid labels are under test. Chemical or colourimetric systems (using biotin-labelled nucleosides and alkaline phosphatase or fluorescein-conjugated avidin) assayed in liquid suspension or when dotted onto nitrocellulose membranes seem appreciably less sensitive than radiolabelled probes. However, experience with viruses of veterinary significance (e.g. bluetongue affecting sheep and cattle) suggests that in situ hybridization (Haase et al. 1984) conveniently and specifically allows the detection of viral genomes in tissues or blood exposed to biotinylated copy DNA followed by avidin-fluorescein or strept-avidin-horseradish peroxidase (Roy et al. 1985). Similar tests could undoubtedly be developed for use with plants even though the composition of the latter may present difficulties.

Double stranded RNA

Some viruses have dsRNA genomes and, during replication, others produce dsRNA. Although too labour intensive for large scale routine use, the extraction from plants and characterization by gel electrophoresis of dsRNA is technically simple and it was suspected that detection of dsRNA of appropriate size might provide insight into the causes of diseases of unknown aetiology (e.g. little cherry, lettuce big vein, June yellows of strawberry, blackcurrant reversion) and for which no reliable assay is available (Dodds et al. 1984). Additionally, it was expected that detection of dsRNA molecules would improve awareness of latent infections in apparently healthy plants because any suspect dsRNA species could be labelled directly or after cloning DNA copies of them in bacteria, thereby facilitating their routine use in ELISA-based systems or in nucleic acid hybridization assays. However, surprisingly large quantities of discrete dsRNAs have been discovered in apparently healthy plants of several species perhaps indicating the presence of unsuspected pathogens as well as symbionts and commensal agents: phylloplane fungi are a likely source of confusion because some mycoviruses have dsRNA genomes (see Matthews 1983). Furthermore, the association of specific dsRNA with disease has not been constant and unexpected seasonal fluctuations in the amounts of virus-specific dsRNA extractable from plants have been revealed by current investigations. Consequently, great caution is needed when interpreting data implying the presence or absence of dsRNA; questionable positives and false negatives are not uncommon.

CONCLUSION

The sensitive and reliable ELISA or nucleic acid hybridization systems that are capable of yielding quantitative data are typically used merely to indicate the presence or absence of a virus. The biological significance of measured virus concentrations is largely unknown and, at a time when the cadre of broadly based virus pathologists is diminishing at an unprecidented rate, the rectification of this ignorance will be delayed. There are likely to be critical threshold concentrations below which the probability of virus acquisition by vectors declines rapidly and with little detriment to marketable yield (e.g. Skaria et al. 1985). The most sensitive detection systems may be misinterpreted to discriminate against the irrelevant or indeed the beneficial viruses that diminish the palatability of plants to herbivores (Gibbs 1980). Arguably the most sensitive and least selective methods are appropriate when testing small numbers of 'mother' plants. Mass indexing of 'certified' crops can sometimes tolerate a diminished standard of stringency. Even if the specificity of some ELISA systems may mean that a few infected plants are missed by such tests, the convenience of ELISA

procedures allows a greater sample of a crop to be surveyed. However, the benefits in terms of apparent efficiency must be measured against the possibility that virus serotypes which escape detection will, sooner or later, become more common and perhaps present new threats.

ACKNOWLEDGEMENTS

I am grateful to Miss Gail Davies for typing.

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METHODS OF PRODUCING, AND SOME BENEFITS OF GROWING, VIRUS-FREE PLANTS

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ABSTRACT

Current procedures for producing virus- and viroid-free plants of vegetatively-propagated crops by meristem-tip culture, thermotherapy and/or chemotherapy are briefly reviewed. Studies to determine the effects of virus-infection on the growth and yield of annual, perennial and vegetatively-propagated crops have shown that virus-free plants are usually more productive, have greater resistance to fungal pathogens and, in some leguminous species, develop larger and more numerous Rhizobium-induced nodules.

INTRODUCTION

The deleterious effects of virus-infection in many annual and biennial crops can be minimised by the use of virus-free seed, preferably of resistant and/or tolerant cultivars, elimination of foci of infection and control of vectors or other mode of spread. With totally-infected cultivars of vegetatively propagated crops, however, it is first necessary to obtain virus-free plants by meristem tip culture, thermotherapy and/or chemotherapy, and to grow plants under conditions in which reinfection is minimised or totally prevented. I review briefly procedures used to obtain virus- and viroid-free plants of vegetatively-propagated species, and the benefits of growing such healthy plants.

PRODUCTION OF VIRUS- AND VIROID-FREE PLANTS

Meristem-tip culture

Since this procedure was first used by Morel & Martin (1952) to eliminate viruses from dahlias, the procedure has been used to obtain virus-free plants of numerous vegetatively-propagated species (Quak 1977, Walkey 1980b). Callus (Svobodva 1965, Mori 1977, Hansen & Hildebrandt 1966), protoplasts (Shepard 1975) and reproductive tissues (Bitters et al. 1972, Navarro & Juarez 1977, Walkey et al. 1977) have also been used for this purpose. Experience during the past three decades or so, however, has shown that viruses are most effectively eliminated by meristem tip-culture (Quak 1961 & 1977, Walkey 1978); such explants regenerate more quickly than those from other sources and, probably because meristemmatic cells are uniformly diploid (Murashige 1974), they are genetically stable. Meristem-tip culture, the requirements for which have been well reviewed (Murashige 1974, de Fossard 1976), has been used for the production of virus-free plants of numerous species (Walkey 1980b).

Some meristem tips may be free of virus; others, however, are infected but virus is inactivated during culture (Hollings & Stone 1964, Walkey et al. 1969, Mori 1977, Krylova 1973), possibly due to effective inhibition of virus replication induced by wounding (Mellor & Stace-Smith 1977).

Chemotherapy

Many substances are known to suppress virus multiplication and/or development of virus-induced leaf symptoms, but few are known to eradicate viruses from plant tissues (Tomlinson, Faithfull & Ward 1976, Tomlinson 1981, Dawson 1984). It has been shown recently, however, that

50-100 mg/litre of Virazole (Ribavirin; 1-B-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) in culture media sometimes inactivates virus in cultured meristem tips; Virazole, probably by inhibiting virus synthesis (Maugh 1976), has been used to eliminate several viruses including potato virus X from potato explants (Shepard 1977, Klein & Livingston 1982), cucumber mosaic virus from Nicotiana rustica (Simpkins, Walkey & Neely 1981), potato Y and cucumber mosaic viruses from N. tabacum cv. Xanthi-nc (Cassells & Long 1980), ullucus mild mottle virus from Ullucus tuberosus (Stone 1982), lily symptomless and tulip breaking viruses from Lilium longiflorum (Blom-Barnhoorn & van Aartrijk 1985) and apple chlorotic leafspot virus from apple (Hansen & Lane 1985).

Although neither Virazole nor phosphonoacetic acid eliminated chrysanthemum stunt viroid from meristem tips of chrysanthemums cv. Bonnie Jean, amantadine did so when added (50-100 mg/litre) as a supplement to the culture medium; 10-15% of plantlets so produced at 24°C were viroid-free (Horst & Cohen, 1980). The inhibitory mechanism of amantadine on viroid replication has yet to be elucidated; nevertheless, as with animal viruses (Hoffman et al. 1965, Skehel et al. 1977), amantadine probably inhibits RNA transcription.

Thermotherapy

Thermotherapy has been used to obtain virus-free plants of numerous infected mother stocks, but has been especially effective in eliminating viruses from perennial fruit crops (Nyland & Goheen 1969).

Thermotherapy combined with meristem-tip culture has also been used to eradicate viruses from herbaceous species; for example, cucumber mosaic and alfalfa mosaic viruses were eliminated from Nicotiana rustica by growing meristemmatic tissues in culture for either 45 days at 30°C or 9 days at 45°C (Walkey & Cooper 1975, Walkey 1976). Cycles of alternating high and low temperatures, by minimising cellular damage attributable to continuous high temperature treatment, have also proved effective for obtaining virus-free plants of other species including potato (Larsen 1966), N. rustica (Walkey & Freeman 1977) and narcissus (Brunt, Stone & Phillips 1984). Potato viruses A and Y can also be eliminated from potato by retaining cultures at continuous low temperature (5°C) instead of continuous high temperature or alternating temperatures (Moskovets et al. 1973). Similarly, by growing meristem-tip cultures continuously at 5°C potato spindle tuber viroid was eliminated from potato (Lizarraga 1980), and chrysanthemum stunt and chrysanthemum chlorotic mottle viroids from chrysanthemum (Paludan 1985). Although not yet tested, these latter results suggest that plants might be freed of viroids more effectively by growing meristems at 5°C in media containing amantadine or other anti-metabolites.

EFFECTS OF VIRUS-INFECTION ON PLANT GROWTH AND YIELD

Viruses can have diverse effects on their hosts. A few are lethal, many seriously reduce the quality of the harvested product, and others so obviously debilitate plants that epidemics in field crops are immediately apparent and readily detected by aerial photography (Colwell 1956, Hooper 1978, Bar-Joseph, Roistacher & Garnsey 1983). The majority of viruses, however, cause substantial but unspectacular crop losses.

The effects of infection on productivity, especially in vegetatively-propagated species and in mixed perennial crops, are difficult to establish because of natural variations in seasonal and geographical factors,

virulence of viruses and relative abundance of vectors. Nevertheless, many disease loss assessments have been made, and some representative examples are now considered.

Annual crops

Viruses infecting annual crops, especially those transmitted in the persistent or non-persistent manner by aphids or by fungi can severely reduce the yield of annual crops. Thus, early infection with severe strains of barley yellow dwarf virus can result in almost total failure of cereal crops, although annual losses in countries such as the UK, New Zealand and Canada are usually c. 10% (Doodson & Saunders 1970, Plumb 1983). Sugarbeet yellows viruses also cause significant yield losses of sugarbeet, with yields being reduced by 4.5% for each week that plants have been infected (Watson, Watson & Hull 1946).

Turnip mosaic and cauliflower mosaic viruses can also seriously affect oilseed rape plants, especially those of some recently introduced cultivars (Walsh & Tomlinson 1985). Both viruses, like many others infecting annual crops, reduce the seed yield of infected plants by inducing the production of fewer and/or smaller seeds. The germination of seed from virus-infected rape plants was significantly lower after storage for 1 year (Walsh & Tomlinson 1985).

Many other aphid-borne viruses severely reduce yields of diverse crops. Although too numerous to consider here, some such as beet western yellows can be particularly damaging in lettuce and oilseed rape (Gilligan et al. 1980, Tomlinson 1972).

Of fungal-transmitted viruses, barley yellow mosaic and beet necrotic yellow vein viruses can severely reduce the yields of susceptible crop cultivars.

Perennial crops

Viruses often significantly reduce the growth and yield of fruit crops (Cropley 1979). Some such as cherry leaf roll virus can kill branches or whole cherry trees, and nematode-borne viruses and complexes of aphid-borne viruses severely debilitate raspberries and strawberries. Although viruses commonly infecting apple, cherry, pear and plum trees have less spectacular effects, they can also reduce yield and cause uneven growth of trees and failure of bud grafts (Posnette & Cropley 1956, 1959, 1965, 1973 & 1970, Posnette, Cropley & Swait 1968, Campbell et al. 1978). Some, however, such as cherry raspleaf (European) virus severely reduce yields of sweet cherry trees; apple mosaic virus reduces crops of apple cvs Allington Pippin and Cox's Orange by c. 30%, apple chlorotic leafspot virus those of pears by up to 40% and viruses of plums by 23-82% (Posnette & Cropley 1959, 1970 & 1973, Campbell et al. 1978). Some viruses also adversely affect the quality of fruit; several viruses of apples cause superficial blemishes and cracking of fruits, raspberry bushy dwarf virus can cause "crumbly" fruits in raspberry and plum pox virus causes unsightly markings of infected plums which have a reduced sugar content and poor flavour (Cropley 1979). There are a few anomolous and unexplained effects of viruses on fruit trees. Thus, cherry cv. Merton Heart crops more heavily when infected with some viruses (Posnette & Cropley 1970) and the Italian prune cv. Richards Early yields more in the USA when infected with prunus necrotic ringspot virus (Helton 1974).

Virus infection also greatly decreases the yield of hops. Prunus necrotic ringspot virus, although often inducing no conspicuous leaf symptoms, can cause c. 20% loss of brewing material, and yield losses of 30-75% occur with severe diseases (nettlehead, bare bine and split leaf blotch) associated with arabis mosaic (Neve 1979).

PNRSV can also have hitherto unsuspected deleterious effects on roses. In cv. Fragrant Cloud, it delayed onset of flowering, reduced the size and number of blooms, increased the number of deformed flowers, caused early autumnal leaf fall, loss of vigour and production of many sub-standard bushes (Thomas 1982). Comparisons with virus-free plants have also shown that arabis mosaic and strawberry latent ringspot viruses seriously debilitate field-grown maiden rose bushes of cv. Fragrant Cloud (Thomas 1984).

Virus-free rhubarb plants in preliminary trials also yielded significantly more than infected plants (Walkey & Cooper 1972). In later tests, however, virus-free plants gave a higher yield of rhubarb harvested for canning, but not of forced or early pulled crops (Walkey et al. 1981). The cold requirement to break dormancy is apparently greater in healthy than infected plants (Walkey 1980a).

It has also long been appreciated that virus-infection can severely affect pasture crops. In the USA, co-infection with alfalfa mosaic and bean yellow mosaic viruses reduced forage yields of white clover cultivars by 23-55%, seed yields by 29-54% (Kreitlow et al. 1957, Kreitlow & Hunt 1958) and their longevity from 10-20 years to 3-6 years (Barnett & Diachun 1984). Bean yellow mosaic virus has similar effects in red clover (Goth & Wilcoxson 1962). Forage legume crops throughout the world are probably similarly affected.

Viruses also seriously affect the growth and yield of grasses; thus, cocksfoot mottle virus can have deleterious effects on cocksfoot (Catherall & Griffiths 1966) and ryegrass mosaic and barley yellow dwarf viruses can reduce the yield of Italian and perennial ryegrasses by up to 30% (Catherall 1966, A'Brook & Heard 1975, McMillan & Holmes 1980). The effects of viruses on pasture grasses, especially in mixed swards, is complex because adjacent plants can compensate for the death or severe effects on infected plants (e.g. Catherall 1966, Catherall & Griffiths 1966).

Vegetatively propagated crops

The deleterious effects of viruses in vegetatively propagated crops often remain unrecognised until virus-free plants are available for comparative tests. A classical example is that of King Edward potatoes which, when free of potato virus S (the cause of paracrinkle disease) yielded c. 10% more potatoes which were more uniform in size and shape (Kassanis 1965).

As implied by its name, lily symptomless virus was earlier thought to have little adverse effect on infected lilies. Virus free plants of cv. Enchantment were found at maturity to be two-thirds taller, to have 33% more leaf cover and with only one fifth of the lower stem without leaves; moreover, such plants had twice the number of flowers which were 12% larger. Flowering of virus-free plants, however, was delayed by c. 6 days and, when grown in pots, required 2-3 times more growth retardant (Menhenett & Hanks 1982).

In small scale trials in the Isles of Scilly, virus-free plants of Narcissus tazetta cv. Grand Soleil d'Or grew much more vigorously than infected plants and produced flowers significantly better in quality; they produced almost twice the number of flower stems and flowers and, after 3 years, about four times the number and fresh weight of bulbs (Stone, Brunt and Hollings 1978).

Bulbous irises free of iris mild mosaic, iris severe mosaic and/or narcissus latent viruses grow much more vigorously than infected plants. In comparisons using cv. Wedgwood, 50 virus-free bulbs (410g total weight) after one year produced 200 bulbs (1893g total weight), whereas 50 similar but infected bulbs produced 181 bulbs (972g total weight). The greater weight of the virus-free stock was largely due to the production of a larger bulbs (A Thompsett, unpublished data).

INTERACTION WITH MICRO-ORGANISMS

There is much evidence that, with a few notable exceptions, virus-infection generally increases the susceptibility of plants to fungal pathogens. Other viruses, however, reduce nodulation by Rhizobium in leguminous species.

Fungal pathogens

Peas infected with bean yellow mosaic virus are more vulnerable to root rots induced by Fusarium species (Kvicala 1966), probably because their roots exude more nutrients and thus induce rapid growth of the pathogen (Beute & Lockwood 1968). Possibly for similar reasons, maize dwarf mosaic virus (MDMV) predisposes maize plants to infection by Fusarium moniliforme (Futrell & Scott 1969), and by Giberella zeae and Helminthosporium pedicellatum (Tu & Ford 1971). Similarly it has long been known that, in contrast to uninfected plants, potatoes infected with potato virus Y are more severely damaged by Phytophthora infestans (Hooker & Fronek 1961). Similarly, greater amounts of phytotoxic mycotoxin were produced by H. maydis in MDMV-infected maize and sorghum than in virus-free plants (Beniwal & Goudanskas 1972). Sugarcane mosaic virus and Pythium graminicola together had a greater effect than either alone in sugarcane (Koike & Young 1970). Infection of oats by barley yellow dwarf virus (BYDV) predisposes plants to leaf blotch disease (Comeau & Peletier 1976), and both barley and oats are more severely affected when infected by both BYDV and the powdery mildew fungus (Potter 1980). Ryegrass mosaic virus and Fusarium nivale also react synergistically in perennial and Italian ryegrasses and, in addition to severely debilitating infected plants, increase their susceptibility to frost damage (McMillan & Holmes 1980).

Although there are many other reports of virus-infection inducing increased susceptibility to fungal pathogens (Watson 1964, Chant et al. 1984, Denis & Elliott 1967, Fantino et al. 1983, Nitzany et al. 1972, Pieczaka & Zitter 1981, Powell 1979, Gbaja & Chant 1982, 1983, 1984 & 1985), there are a few reports of enhanced resistance resulting from virus-infection; thus some cucurbitaceous species infected with watermelon mosaic virus are less susceptible and/or less severely damaged by F. solani f. sp. cucurbitaceae (Diaz-Polanco 1969) or Sphaerotheca fuligena (Besada 1978), and the resistance of soybeans to Cephalosporium gregatum was apparently increased by prior infection of plants with soybean mosaic virus (Tashibana & Card 1972).

Nodule-forming bacteria

Some viruses severely reduce nodulation in infected leguminous species, and thus severely impair their ability to fix nitrogen. Thus, infection by soybean mosaic virus reduced the number, size and weight of nodules in soybean by up to 81%, the extent of the reduction mainly depending on earliness of virus-infection (Tu et al. 1969); moreover, infected plants were less susceptible to Rhizobium, and nodules that were formed functioned inefficiently (Tu et al. 1970). Alfalfa mosaic, clover yellow vein and peanut stunt viruses each caused the formation of fewer, smaller, mis-shapen and discoloured nodules in white clover (Gibson et al. 1981, Smith & Gibson 1960), although white clover mosaic virus reduced their number, but not their size or shape (Guy et al. 1980).

PROGNOSIS

The elimination of viruses from vegetatively propagated planting stock has already resulted in significant improvements in the yield and/or quality of many crops thoughout the world. Such improved planting stock will undoubtedly continue to be required for its greater productivity, as starting material for rapid methods of micropropagation and to facilitate the international exchange of germplasm.

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PREVENTION OF VIRUS SPREAD

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ABSTRACT

Preserving the health of virus-free stocks of plants depends entirely on preventative measures which either suppress vector activity or decrease the availability of plant virus sources. The forms of control applicable depend on the ecology of the virus; important features are the type of vector, the persistence of the virus in the vector and the variety and frequency of occurrence of virus hosts. Transmission of viruses by aphids can be prevented in several ways, including the use of insecticides, oil emulsion sprays, reflective mulches and barrier crops and screens. Diseases caused by nematode-borne viruses are best controlled by avoiding or eliminating virus sources, as too are diseases caused by viruses that spread, or mainly spread, without vectors. A strategy of over-protection, using combinations of complementary control measures, is recommended for the production of virus-free stock.

INTRODUCTION

Although different plant viruses are transmitted in many different ways, most individual viruses are transmitted in only one main way. Under the special conditions of crop culture a few viruses spread mainly, if not only, by contact between healthy plants and infected plants or virus contaminated surfaces but in natural plant communities most viruses depend on a specific organism (the vector) for transfer from one plant to another. However, the natural mode of spread of some viruses, such as potexviruses and tombusviruses, is still unexplained. Vectors of plant viruses occur in several taxonomic groups including Insecta, Acarina (mites), Nematoda and Fungi but in temperate climates most viruses that cause economically important diseases are transmitted either by aphids or by ectoparasitic soil inhabiting nematodes. Understandably, many investigations have tested ways of preventing the spread of viruses by these vectors and it is on these control measures that this paper will mainly concentrate.

Virus survival has two main rudiments: the perennation of virus sources and a means of spread. As there is no direct means of eradicating virus in its vector or host plant on a large scale, control can only be accomplished by obstructing one or more of the series of events involved in virus survival and spread. The form of control depends on many factors, which include the range of plant hosts of the virus, their spatial and temporal distribution, the type of vector and its distribution, and the kind of association of the virus with its vector. Often control is achieved by disrupting the process of transmission from plant to plant and therefore it is convenient to consider control measures in relation to the main modes of virus transmission.

TRANSMISSION BY APHIDS

Types of transmission

The length of time a virus persists in the aphid largely determines the possible kinds of control measure likely to be successful. Three main

types of association between virus and vector are recognised. Non-persistent viruses are characterised by the aphid acquiring virus during initial probes of about a minute or less on the infected plant and can be transmitted to healthy plants immediately. Retention of the virus is brief and is often only a few minutes to an hour. Typically the specificity between the virus and the aphid is not well developed and many aphid species, including some not normally found on the virus infected hosts, may transmit. With persistent viruses, by contrast, specificity between the virus and aphid is well developed and transmission is usually by only one or a few aphid species. Following acquisition of the virus there is a latent period which, depending on the virus, can last for several hours or even several days and during which the aphid is not infective. Once infective the aphid remains so for 2-3 weeks or for life, even when the aphid moults during this period. A third category, semi-persistent viruses, can persist in aphids for an intermediate period, usually a few days, but do not pass through the moult.

Use of insecticides

Insecticides, such as organophosphorus and carbamate compounds, have not been as generally successful in controlling aphids as virus vectors as they have been in controlling aphids as pests, for which they were designed. The two main reasons why an insecticide may be ineffective in preventing virus spread are first it may not kill the aphid before it transmits the virus and secondly it may not decrease the number of aphids sufficiently. Thus predictably such insecticides have been useful in decreasing the incidence of infection by persistent and semi-persistent viruses because the insecticide acts before the aphid can acquire and transmit virus. For example, insecticides have been used effectively to diminish the spread of beet yellows virus in sugar beet plantings and of potato leafroll virus in potato, but their effect is mainly on spread from virus sources within the crop and they provide less protection from virus introduced by aphids from sources outside the crop. In contrast such insecticides have not controlled the spread of non-persistent viruses within crops and indeed examples are known in which the incidence of infection was increased because aphid behaviour was affected in such a way as to favour transmission of virus from plant to plant. Pyrethroid insecticides, however, kill aphids very quickly and offer promise for the control of both non-persistently and persistently transmitted viruses (see accompanying paper by R.W. Gibson, G.R. Cayley & R.M. Perrin).

Application of oil emulsion sprays

Since Bradley et al. (1962) discovered that transmission of potato virus Y by Myzus persicae was impeded by coating the source plant or the test plant with mineral oil, the transmission of many other viruses has been shown to be affected similarly. Several reports indicated that the effect applied to all non-persistent viruses tested and also to the semi-persistent beet yellows virus (Vanderveken & Semal 1966), whereas the transmission of persistent viruses such as pea enation mosaic (Vanderveken 1968) and potato leafroll (Hein 1971) appeared to be unaffected. However, more recently Zitter and Everett (1979) found that the spread of tomato yellows luteovirus in the field was reduced by mineral oil sprays, suggesting that the effect on persistent viruses needs to be re-appraised.

Although many experiments have been done on the use of oils with a range of viruses and crops, and the potential for the control of spread of

non-persistent viruses is well established, nevertheless instances of the commercial application of oil sprays for controlling virus spread are few. One reason is the apparent lack of interest by agricultural chemical manufacturers in developing suitable oil formulations. With the exception of JMS Stylet-Oil (Zitter & Ozaki 1978) most of the oils examined are those produced for other purposes, may not be widely available and in many instances produce unacceptable phytotoxic effects.

The mechanism by which mineral oils inhibit acquisition and inoculation of virus by the vector aphid, the first more effectively than the last, is not known. However, some properties of an oil associated with efficacy have been identified. Thus de Wijs et al. (1979) and de Wijs (1980) concluded that the viscosity gravity constant (VGC) and the viscosity were the two most important properties and that mineral oils with a VGC between 0.790 and 0.819 and a viscosity between 12 and 30 cST at 37°C (66-150 Saybold Universal Seconds (SUS)) are optimal for virus control. In field trials, Simons & Zitter (1980) and Simons (1982) found that an oil of 70 SUS viscosity was usually more effective than those of 60 and 110 SUS and that emulsifier, spray pressure and nozzle orifice size also had effects. A major drawback with oil sprays has been their phytotoxicity. This has been related to the percentage unsulphonated residue (not less than 95% is required to avoid phytotoxicity), viscosity and concentration of the oil (de Wijs 1980). Different plant species, however, vary greatly in their susceptibility to damage and different oils seem to rank similarly for phytotoxicity to different plant species (Asjes personal communication).

Because winged aphids usually alight on the outer parts of the leaf canopy of a crop and then usually probe the upper surface of leaves, it may not be important to cover all other plant surfaces with oil. Oils have been reported to persist on leaves for 10-14 days (Simons et al. 1977) but the frequency of applications needed will be determined by the rate of production of new foliage.

Although a complex set of interactions is involved in selecting a suitable oil and developing a regime for the use of oil sprays on a crop, the potential of the method for controlling non-persistent viruses would seem to justify the investment. Examples of successful adoption into standard practice may be few but, as in the control of tulip breaking virus in lily, the method is clearly effective. (Asjes 1984).

Use of reflective mulches

During the host-seeking stage of their dispersal or migratory flight, aphids are repelled by short wavelength light (Johnson 1969). Kring (1964) was the first to suggest exploiting this behavioural feature by using aluminium foil laid between rows of plants. Since then there have been numerous reports of decreasing the incidence of non-persistent viruses by using reflective mulches. For example Nawrocka et al. (1975) found that either aluminium foil or black plastic decreased the spread of cucumber mosaic virus in field grown lettuce from 40% to 2.5%. For effective repulsion a ground cover of not less than 50% is recommended and in a comparison of plastic sheets of different colours Jones & Chapman (1968) found that yellow was most attractive followed in order by pink, green, red and black whereas white, orange, light blue, aluminium foil and dark blue attracted fewest aphids. The two main disadvantages of this method of control is the deterioration of reflectance after the first few weeks in

the field (Loebenstein et al. 1975) and the decrease in reflectant area as the plant canopy enlarges. Nevertheless this control measure may still be useful because it will be most efficient in the early stages of plant growth which is when plants are most susceptible to virus infection.

An unusual variant of this technique is to surround beds of plants with vertically hanging sticky sheets of yellow polyethylene which attract and trap migrant aphids. By this means the incidence of aphid-borne viruses in pepper grown in Israel was decreased by 50-80% Cohen & Marco (1973).

Use of barrier crops and screens

Effective protection of sugar beet seedlings in Britain against virus yellows was obtained by growing them between rows of mustard or barley even though mustard is a host for the vector aphids (Heathcote 1968). Similarly, Jenkinson (1955) reported that the spread of cauliflower mosaic to seed beds of cauliflower was decreased by using kale or barley barrier crops even when the beds were 4.5 metres from infected plants. Such plant barriers probably function in two ways:- by intercepting the migrant aphids which probe and loose their charge of virus before moving on to the protected plants, and by shielding the protected plants from alighting aphids, which may fly off again without having reached the plants enclosed by the barrier.

Polyethylene nets (as used in windbreaks) can also prevent virus spread and were used successfully to restrict infection of Lilium formosanum by lily symptomless and tulip breaking viruses in experimental plots (e.g. Mowat & Woodford 1976). Such plastic screens have the advantage of not competing with the protected crop for nutrients and light and possibly aphids may even loose their virus charge by attempting to probe the plastic, although this has not been established. However, barriers can present problems by interfering with cultivation practices.

Another way of using netting is to camouflage plants and this method has been used in Israel to protect Capsicum annuum and potato plants (Cohen 1981). White polyethylene nets (mesh 3 mm) placed above and around plots decreased the incidence of cucumber mosaic virus and potato virus Y in peppers about 10-fold. Potato plants covered in a similar way with a coarser white net (mesh 10 x 3 mm) largely prevented the spread of potato leafroll virus. These materials may have a role as an alternative to the use of an insect-proof gauze-house under conditions where there is only a moderate risk of spread.

Limiting the influence of virus sources

The prospects for preventing virus spread through restricting virus sources are best for viruses with a limited host range that does not include any commonly occurring wild plant species. Effective control may then be achieved by propagating stocks in areas distant from those where the crop is grown commercially, as for example was previously done with beet stecklings in Britain, or by imposing an effective spatial isolation from infected crops as in seed potato production. The isolation distance required to give effective protection depends on many factors, cannot be predicted precisely and has to be determined empirically. Predictably, however, control by spatial isolation from viruses of the persistent type will be more difficult than for non-persistent viruses. Nevertheless the

occasional introduction of a non-persistent virus from a distant source by a migrant aphid must always be considered a possibility. Virus infected perennial wild hosts are an obvious problem as are infected annual weeds which overwinter or in which viruses are transmitted through the seed. Rigorous weed control applied within and around the crop can, where practicable, help to control viruses of the non-persistent type but in itself can be expected to have little influence on the introduction of persistent viruses and their subsequent spread in the crop itself.

TRANSMISSION BY NEMATODES

Nematodes involved

Vector nematodes belong to only four genera: *Longidorus* and *Xiphinema*, species of which transmit nepoviruses (Murant 1981) and *Trichodorus* and *Paratrichodorus*, species of which transmit tobraviruses (Harrison & Robinson 1978). Virus persistence is least in *Longidorus* species (up to 12 weeks for tomato black ring virus), longer in *Xiphinema* species (c. 8 months for arabis mosaic virus) and longest in trichidorid nematodes (up to 1 year for tobacco rattle virus). The relative immobility of these vectors has an important bearing on the approach to controlling the spread of nematode-borne viruses. Thus, unlike aerial vectors the nematodes are not an important means of virus dispersal and present a stationary target for detection and eradication. Dissemination of nematode-borne viruses therefore depends on the dispersal of plant sources of virus, that is infected vegetative planting material and seed (especially weed seed).

Use of nematicides

Control of nematode-borne viruses by applying chemicals to the soil has been obtained with pre-plant applications of the fumigants dichloropropene, methyl bromide and methyl isothiocyanate (from dazomet prills). For example dichloropropane-dichloropropene (D-D) killed 99% of *Xiphinema diversicaudatum* and decreased the incidence of arabis mosaic virus in strawberry from 78% to 1% (Harrison *et al*. 1963). Similarly D-D killed 99% of trichodorid nematodes and reduced the incidence of TRV in potato from 75% to 1% (Cooper & Thomas 1971). However, effective as these fumigants are in killing nematodes the end result may be only a partial and temporary reduction of virus incidence as for example was found in attempts to control arabis mosaic virus in *Lilium tigrinum splendens* (Asjes & Segers 1983). This is because some nematode vectors survive fumigation and virus sources are sustained in virus-infected volunteer plants from stem bulbils shed by the previous crop. Non-fumigant systemic nematicides such as aldicarb and oxamyl have also been used. These have added advantages in that they are also insecticidal and are not phytotoxic. Unlike the fumigants these chemicals do not necessarily kill the nematodes but prevent transmission by inhibiting feeding. Although this effect is temporary, and they have only limited persistence, nevertheless both oxamyl and aldicarb decreased the incidence of tobacco rattle virus in potato tubers from 19% to 3-4% in field trials (Alphey *et al*. 1975). Another chemical which may also interfere with feeding, but also kills the nematodes, is the fungicide pentachloronitrobenzene. Applied before planting it decreased the numbers of *Longidorus elongatus* by 98% and protected strawberry plants from infection by tomato black ring and raspberry ringspot viruses for four years (Murant & Taylor, 1965). Although effective in decreasing virus spread this chemical is degraded to form compounds that are phytotoxic to some raspberry cultivars and cause abnormalities in root crops.

Avoidance of virus sources

In principle control should be readily obtained by maintaining freedom from host plants for a period exceeding the persistence of the virus in the nematode vector. This is most promising for the Longidorus-transmitted viruses because of their relatively short persistence in the vector. Some success with tomato black ring and raspberry ringspot viruses has been obtained by this means in field trials (Taylor & Murant 1968) but has not become an established practice in the industry. In contrast the use of a 2-year fallow after removal of an infected hop planting has become an adopted procedure in the control of the hop strain of arabis mosaic virus (McNamara et al. 1973). Weed control, however, can have counter-productive effects where the crop continues to be grown. Thus Cooper & Harrison (1973) found that the incidence of infection of tobacco rattle virus in potatoes was greater in plots kept weed-free for 2 years than in weed-infested plots, presumably because more viruliferous nematodes fed on potato plants when weeds were not available.

Although widespread in their distribution (Taylor & Brown 1976, Alphey & Boag 1976) all vector nematodes are not present in all soils, nor are all vector populations viruliferous (about 1% of Longidorus elongatus populations were found to be carrying tomato black ring virus, c. 5% of Xiphinema diversicaudatum populations were carrying arabis mosaic (Taylor & Brown 1976) and 68% of trichidorid populations in freely drained podsols were carrying tobacco rattle virus (Cooper 1971). Avoidance of infection is therefore possible and in the Scottish virus-tested narcissus scheme, the risk of infection by arabis mosaic and strawberry latent ringspot viruses is eliminated by growing high-grade virus-tested stocks north of the river Tay, the northern limit of their vector X. diversicaudatum. In addition, sites are selected for freedom from nematodes carrying tomato black ring, raspberry ringspot and tobacco rattle viruses by soil-sampling to detect vector nematodes and by a plant bait test of the samples to determine whether the vectors are viruliferous. Although the patchy distribution of nematode vectors in fields (Boag & Topham 1984) would suggest that soil sampling of sites for vector nematodes may not always be dependable, nevertheless this approach has been successful when applied to the propagation of virus-tested stocks of fruit plants and hops in England (Cotten 1979).

TRANSMISSION WITHOUT VECTORS

Spread by mechanical means

For a few viruses, mechanical transmission is the main means of spread into and within crops. Thus tobacco mosaic virus can infect tomato plants from contaminated implements, hands and clothes, and once established can spread to healthy plants by contact. Similarly, potato virus X can be introduced into virus-free crops by implements and even on clothing and on fur of animals contaminated with virus from an infected crop (Todd 1958). Prevention of spread of such viruses depends on strict management to avoid contact of operators and tools with virus sources, the use of uncontaminated overalls and the decontamination (with detergent or trisodium phosphate) of tools, hands and other surfaces that may be contaminated with virus. Although particles of mechanically transmitted viruses typically occur in high concentrations in plant tissue, this cannot be assumed to be the only prerequisite for such transmission. For example, potexviruses and most tombusviruses have no known biological vector and it has often been assumed or speculated that members of these virus groups spread by plant contact or by some mechanical means. Yet two such viruses, narcissus mosaic and

narcissus tip necrosis, which produce about 100 µg of particles per g of leaf tissue, appear not to spread by plant contact, handling or flower picking (Asjes 1972, Mowat 1980). Thus in the management of virus-free narcissus stocks, and contrary to earlier speculations, these viruses do not present a problem for control comparable to that presented by potato virus X or tobacco mosaic virus.

Spread through seed and pollen

Transmission of viruses through seed was formerly considered an unusual occurrence but there are now over 100 viruses or virus-like infections reported to be seed-borne in infected plants and seed can also be infected with many of these viruses introduced via infected pollen (Mandahar 1981). Except for cryptic viruses, for which seed transmission is as yet the only known means of perennation, the embryo-infecting viruses all have an additional means of spread. Nevertheless seed transmission is a major obstacle to maintaining the health of virus-free material of some crops because the occurrence of virus sources within the crop will result in rapid spread to neighbouring healthy plants when vectors are active. There is no generally effective means of eliminating viruses from infected embryos and control largely depends on minimizing the amount of primary virus sources within a crop by screening seed-producing plants and seed samples. For example although lettuce mosaic potyvirus, a non-persistent aphid-borne virus, is usually transmitted through less than 5% of seeds of infected lettuce plants (Kassanis 1947, Broadbent et al. 1951) seed transmission is a major factor in its spread in England and California. Indeed to obtain marketable crops of lettuce in California a sample of 30 000 seedlings from seed batches are required to be free of lettuce mosaic virus (Grogan 1980).

Some viruses can be transmitted through pollen not only to seed but also to the plant pollinated. Such pollen transmitted viruses are common in fruit crops and although the obvious way of controlling spread is by preventing flowering this is hardly an acceptable means of control in commercial crops. However the prevention of flower production in nurseries used for vegetative propagation of virus-tested stocks of these crops is possible and, for example, is routinely practised to control raspberry bushy dwarf virus in raspberry cane nurseries in Scotland.

INTEGRATION AND PRACTICE OF CONTROL METHODS

With extensive knowledge of virus ecology and epidemiology, control measures can be rationally designed and even the time of their application forecast for each growing season. Examples of virus control at this level of sophistication are few but such systems have been evolved for sugar beet in England (Hull 1968, Jepson & Green 1983) and potatoes in the Netherlands (Hille Ris Lambers 1972). Most often, however, decisions have to be taken when knowledge is imperfect, sometimes when the mode of spread is still unknown. Even when some epidemiological information is available, there remains the difficulty in predicting the variation in spread from year to year and from location to location. Obviously the importance of this uncertainty is greater when considering the maintenance of basic virus-free stock where a higher order of protection from infection will be required than would be needed for commercial cropping. In these circumstances the strategy for virus-free stocks is to provide over-protection, to direct control measures at several points of the transmission chain and to use combinations of complementary treatments. For example a combination of oil

spray and barrier crop greatly diminished the spread of non-persistent viruses in lilies compared with the treatments applied individually (Mowat & Woodford 1976) and control of PVY in potato was enhanced by incorporating a pyrethroid with an oil emulsion spray (Gibson & Cayley 1984, Gibson, Cayley & Perrin, this publication). When measures applied against vectors are compounded with control of plant virus sources, for example by spatial isolation from neighbouring infected crops and by use of land free of volunteer plants from previous crops, this will further augment protection. Likewise to prevent infection by nematode-transmitted viruses sites should be selected not only on the basis of a plant bait-test, indicating freedom from viruliferous nematodes, but also, recognising the limitations of the test, a nematicide should be applied as an insurance. However, because the biological background within which many of these control measures act is very complex, a preliminary assessment of their efficacy is advisable to guard against counterproductive effects. For example, weed control during the growing season favoured infection of potato by tobacco rattle virus (Cooper & Harrison 1973) and it is possible that a reflective mulch may so alter the growth of a plant that it may encourage colonisation by insect vectors (Zalom 1981, Wells et al. 1984). Alternatively the control treatment may favour other diseases. Thus a mustard cover crop favoured the development of downy mildew in sugar beet (Heathcote 1968) and oil emulsion sprays may favour the development of Botrytis diseases in tulip crops (Asjes 1983, 1985).

In practice the control measures that can be applied will not only depend on their compatibility with the husbandry of the crop but also on the costs that can be supported by the value of the crop. Often this value will be difficult to determine in advance, with the result that fewer control measures are likely to be applied than is possible and desirable. These costs need to be viewed by growers as a long term investment because as the general health of a commercial crop improves through the introduction of virus-tested stocks so will virus epidemiology be affected, resulting in a decrease in infection pressure and a lessening in the rate of deterioration of commercial stocks. In the final analysis, the adoption of control measures to extend the range of crops for which virus-tested stocks can be made available depends on long-term planning and investment by growers and by research and development organisations. Thus progress will depend not only on the development of new methods of control, such as aphid repellent and feeding-deterring chemicals (e.g. Gibson et al. 1982, Rice et al. 1983), but also on agreement between interested parties on the crops to be selected for improvement by virus-tested schemes and on the co-operation and funding needed to initiate, establish and evaluate the schemes.

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3.
Fungi and Bacteria

Chairman : R. T. Plumb
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SEED AND TRANSPLANT TREATMENTS FOR THE PRODUCTION OF HEALTHY VEGETABLES

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ABSTRACT

Vegetable seeds are sown directly into either field soil or modules from which plants are later transplanted. Freedom from disease is achieved by the production of pathogen-free seeds and by the use of eradicative seed treatments.

Chemicals added to the compost of module systems protect the plants during the propagation stages and this protection may continue after transplanting.

INTRODUCTION

Many modern systems for growing vegetables require a high level of performance from the seeds with ideally each field-sown seed producing a healthy seedling which enables the plants to be established at the correct densities thereby achieving optimum crop performance and yield. A similar requirement can be applied to the raising of transplants where each module (block or cell) should contain a healthy seedling or seedlings.

Although in the past the majority of field vegetables have been direct drilled, ADAS estimates for field vegetables in 1985 (Table 1) show an increased use of hybrid seed, with a movement away from field drilling to the use of modular transplants and a general decrease in the use of bare root transplants.

A high standard of health and vitality is now an important requirement of seeds. The incidence of seed-borne pathogens of vegetables and their control is well documented (Maude 1983, Maude et al. 1984) but changes in transplant production systems may introduce new sources of existing diseases and these may be widely disseminated by the distribution of contaminated plants and soils.

SEED-BORNE DISEASES

Seed-borne fungi and bacteria have their origins in seed production crops. The health status of certain vegetable seeds has been improved by the production of commercial seed stocks abroad in semi arid areas such as the west coast of America, where conditions are not conducive to disease development. Even those procedures however may not exclude the bacterium Pseudomonas phaseolicola (halo-blight of French beans) and the fungus Septoria apiicola (leaf blight of celery) which are capable of causing severe crop losses from initially low levels of inoculum (Maude 1983). The present high cost of producing seeds has resulted in the growing of seed crops in countries where production costs are cheaper but climatic conditions may be more conducive to disease development. As a result, imported seeds of some crops e.g. runner beans have been found with a high incidence of seed-borne P. phaseolicola (Taylor et al. 1984). Seed tests are available for the identification of infected stocks (Taylor et al. 1979) and these can provide seed merchants with information on the disease status enabling them to reject unwanted samples (Maude 1985). Adoption of such tests has been successful in excluding Ascochyta fabae (leaf spot) from

TABLE 1

Trends in crop establishment methods - 1985

Crop	UK area ha	% field drilled	% transplanted: cells	% transplanted: blocks	% transplanted: bare roots	% hybrid seed
Brussels sprouts	11,000	20	6 ↑	3	70 ↘	100
Cauliflowers: Summer/autumn	10,500	1	40 ↑	12	45 ↓	-
Roscoff	2,350	1	5 ↗	0	94 ↘	-
Winter hardy	2,650	0	40 ↗	0	60 ↘	-
Cabbage: Summer/autumn	6,200	13	16 ↑	16	55 ↓	60
Savoy	2,500	10	12 ↗	3	75	90
Winter white	3,000	22 ↘	8 ↑	1	70 ↘	80
Other winter	2,700	30	10 ↗	5	55	55
Spring	7,000	60	4 ↑	2	34	-
Calabrese	1,000	56 ↓	24 ↑	12	8 ↓	90
Sprouting broccoli	500	30	0	2	67	-
Chinese cabbage	220	24	36 ↑	40	0	85
Onions: Bulb - Spring sown	5,700	83 ↘	17 ↗	0	0	40
- Over-wintered	1,000	97	3	0	0	50
Leeks	2,000	42 ↗	18 ↗	10 ↓	30 ↓	-
Celery	950	2	45 ↑	33 ↘	20 ↓	-
Lettuce	6,400	33 ↓	14 ↑	50	3 ↓	-
Sweet corn	1,220	83	4	13 ↗	0	100
Courgettes/marrows	400	10	0	90	0	-
Beetroot	2,900	96	2	2 ↗	0	-

Trend: ↑ Increasing
↗ Slight increase
↘ Slight decrease
↓ Decreasing

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field bean seed stocks (Hewett 1973) and the principle is ripe for application to nuclear stocks of runner beans for the production of commercial seeds free of P. phaseolicola.

However in most cases, these are medium to long-term objectives and seed merchants require more immediate remedies for the seed-borne and soil-borne pathogens attacking seeds and developing seedlings.

SEED TREATMENTS

Vegetable seeds represent a wide range of plant species and thus bear a varied flora of pathogens. As a result, the vegetable seed merchant, has to apply different seed treatments to different seed types in order to eliminate or reduce the seed-borne inoculum to ineffectual levels (Table 2 - Maude 1983).

TABLE 2

Efficacy of curative seed treatments*

Crop and disease	Seed treatment	% range of control	% eradication (mean)	% eradication needed for disease control
Celery - *Septoria*	Hot water	82.8- 99.7	93.3	99.9
	Thiram soak	100.0	100.0	
Onion - *Botrytis*	Benomyl + thiram dusts	99.2	99.2	99.0
Peas - *Ascochyta*	Benzimidazole dust and slurries	98.0-100.0	99.5	94.0-98.0
	Thiram slurry	-	14.0	
Brassica - *Alternaria*	Hot water	95.3-100.0	98.4	90.0
	Thiram soak	52.6- 99.5	89.0	
	Iprodione dust	77.0-100.0	97.8	
	Fenpropimorph slurry	98.0-100.0	99.8	
	Thiram slurry	44.6- 94.9	67.2	
Brassica - *Phoma*	Hot water	61.9-100.0	88.2	99.4-99.99
	Thiram soak	80.0-100.0	90.2	
	Thiabendazole	100.0	100.0	
	Thiabendazole slurry	100.0	100.0	
	Benomyl + thiram slurry or dust	100.0	100.0	
	Iprodione dust	56.0-100.0	91.7	
	Fenpropimorph slurry	85.7-100.0	97.8	
	Thiram slurry	0 -100.0	41.7	

* = based on data from published literature, see Maude (1983) and Maude *et al.* (1984)

Heat treatments

Heat has been applied in wet and dry forms to vegetable seeds to control seed-borne pathogens. The heat balance achieved during treatment is critical and the difference between the temperature which eliminates the pathogen and that which kills the seed host is small. Wet heat is the more effective (Maude 1966a). Aerated steam has been used commercially on flower seeds (Baker 1969, Hall & Taylor 1983); and to some extent against certain vegetable seed pathogens (Maude 1983, Navaratnam et al. 1980). Immersion of seeds for periods of 20-30 min, in hot water, (50°-51°C), has been used in the treatment of fungal (Bant & Storey 1952) and bacterial (Walker 1923) infections of vegetable seeds. Such treatments are attractive in that no chemicals are used but they may adversely affect germination in certain circumstances (Maude 1983); also they may be insufficiently eradicative to reduce inoculum below the minimum threshold level and only allow the throughput of small quantities of seed (Maude 1978).

Cognisant of these disadvantages, merchants only use wet heat treatments on a small scale for specific problems such as the control of some bacterial diseases of vegetable seeds e.g. Xanthomonas campestris infection of brassicas.

Chemical seed soak treatments

Soaking seeds in aqueous chemicals ensured penetration of the seed tissues during imbibition of water thereby eradicating deep-seated infections (Maude 1966b).

Soaking of seeds for 24 h at 30°C in 0.2% aqueous thiram was developed mainly for the treatment of diseases of vegetable seeds (Maude et al. 1969) (Table 2). It was a non-selective treatment acting against a wide range of seed and soil-borne fungi; it was generally more effective than hot water or diethyl mercury phosphate (Dunning & Byford 1973) and, up to 50 kg of seed (maximum 5 kg with hot water treatment) could be treated at one time. The disadvantages were that it was not effective against seed-borne bacteria and the process was lengthy (24 h with an additional 6-12 h drying depending on seed type). The thiram soak treatment now is used commercially in Britain to control Phoma betae in red beet and Septoria apiicola in celery seeds (Table 2).

Keyworth & Howell (1961) successfully eradicated Corynebacterium betae (silvering disease) from red beet by soaking seed clusters for 24 h in aqueous streptomycin sulphate (200 ug/ml). Replacement of the hot water treatment of brassica seeds for the control of X. campestris by antibiotics soaks (Humadayan et al. 1980) was not successful because attempts to antidote their phytotoxic effects with sodium hypochlorite were unreliable.

Seed surface applications of chemicals

Some of the eradicant seed soak treatments were also effective in protecting emerging seedlings from damping-off fungi in the soil. Fungicides such as captan or thiram whose protectant action against these organisms was described by Jacks (1951) have been applied as slurry or dust formulations to peas, beans, carrots, leeks and other vegetable seeds.

Neither fungicide has penetrant action, however, with the later introduction of systemic fungicides such as the benzimidazole-based

compounds, tissue penetration was obtained resulting in the elimination of many seed-borne pathogens such as Ascochyta pisi (Maude & Kyle 1970) and Botrytis allii (Maude & Presly 1977), but not dark-spored Hyphomycetes such as Alternaria and Stemphylium spp. nor the phycomycetous Pythium and Phythophthora spp. The dicarboximide fungicide iprodione and the morpholine fenpropimorph were shown to be active against the Alternaria fungi A. brassicicola and A. brassicae controlling their seed-borne phases in cabbage (Brassica oleracea) (Maude & Humpherson-Jones 1980, Maude et al. 1984). They also eradicated Phoma lingam (canker) from brassica seeds (Maude et al. 1984) and have since been formulated commercially for the treatment of Alternaria and Phoma infections of rapeseed (Maude and Humpherson-Jones 1984).

Although fungicides of benzimidazole, dicarboximide and morpholine origin are effective against a number of fungal seed-borne pathogens of vegetables (Table 2) they are not toxic to Pythium and Phytophthora spp. hence it has been necessary to formulate many of them with broad-spectrum protectant fungicides to extend the range of action, for example, benomyl + thiram (Benlate T) and thiabendazole + captan (Hy-T).

The development of systemic fungicides with specific activity against Pythiaceous and Peronosporaceous fungi (Schwinn 1981, Bruin & Edgington 1983) have made more advanced formulations possible. For example, seed treatments with the acylanaline fungicide metalaxyl controlled Pythium damping-off of peas (Kraft 1982) and protected the young leaves from early attack by Peronospora destructor (Brokenshire 1980).

Formulations of metalaxyl with thiabendazole and of the phosphorate fungicide fosetyl with thiabendazole are commercially available for the control of seed-borne Ascochyta pisi, soil-borne Pythiacous fungi and leaf-borne Peronospora destructor.

Nevertheless, such formulations may still require the addition of a broad-spectrum fungicide to act against non-Phycomycetous soil-borne fungi such as Rhizoctonia solani which cause damping-off and wirestem of certain vegetable seedlings.

Effects of changes in pesticide formulation on seed application techniques

The manufacture of pesticide mixtures containing up to three fungicides, and possibly an insecticide, have promoted the development of liquid formulations with a better retention by seeds and providing a more uniform distribution of the chemical.

Fungicide dusts do not adhere well to treated seeds, (Maude & Presly 1985) slurry formulations are better retained but produce residual pesticide material which may block the drills.

Recently, the possibility of treating seeds by film coating has been explored; this principle involves suspending seeds in a column of warm air followed by coating with quick drying adhesives (Maroglou & Nienow 1985) many of which act as carriers for pesticides. The adhesive dries to a permeable but hard coat which allows the seeds to flow freely in drill machinery.

DISEASES OF VEGETABLE TRANSPLANTS

Crops such as celery, leeks and many of the brassicas (Table 1) were

traditionally produced in soil in frames or beds and then removed as bare root transplants for planting in the field. The soil of the beds was often sterilised to eliminate damping-off organisms and routine fungicide sprays were applied to protect the seedlings and young plants against leaf pathogens. Bare root transplants, of brassicas were dipped in fungicides at lifting to protect them against clubroot (Plasmodiophora brassicae) in field soil (Channon et al. 1965, Buczacki 1973).

Now, however, the use of bare root transplants is declining in favour of module systems of block raised or cell raised transplants (Table 1). The number of vegetable plants raised in cellular trays (modules) rose from a few million in 1981 to 250 million in 1984 (Anon 1985). There were twenty-five plant raising systems available in the UK in 1983 representing a cell/m^2 range of 516 (60 ml/cell) to 4274 cells/m^2 (0.3 ml/cell) (Salter 1985). Increasingly, traditionally field-drilled crops are being provided as transplants as a means of improving crop establishment, uniformity and earliness of production.

These changes present new dangers to the health of transplants and new challenges in disease control.

Effects of modular raising systems on plant diseases

Recently, clubroot infected Brussels sprout plants were discovered in 4.3 cm peat blocks after the main crop had been planted. Test plants grown later in peat blocks of the same origin failed to develop the disease (Buczacki & Stevenson 1983) and the source of origin of the pathogen was not established. Other similar outbreaks have occurred but it has not been proved if the source peat was affected or if the blocks became contaminated on the holdings, nevertheless these reports indicate how a modular transplant system could act as a distribution source for locally restricted soil-borne pathogens.

In 1977-78, outbreaks of lettuce big-vein were reported in field and glasshouse crops over a wide area in Britain. The disease, transmitted by the fungus Olpidium brassicae, occurred in field plants originating from transplants grown in peat blocks. The most serious outbreaks were in re-circulated nutrient solutions, (nutrient film technique - NFT), where the disease affected 99-100% of the plants. It is thought that the plants contracted infection during propagation in the nursery and before transplanting (Tomlinson & Faithfull 1979a).

In this instance, widespread disease outbreaks in field and glasshouse lettuce originated from a single contaminated plant raising source.

DISEASE CONTROL IN MODERN TRANSPLANT RAISING SYSTEMS

Disease-free (within treatment and or testing definitions) seeds should be used to prevent the transmission of fungi such as Septoria apiicola, Alternaria brassicicola and Botrytis allii which could spread rapidly during propagation.

Disinfection of the propagation area also is necessary to prevent the spread of zoosporic fungi disseminated in moisture films amongst block-raised plants. To control the source of big-vein of lettuce peat blocks should be isolated from contaminated soil and debris by placing them on Correx sheets (MAFF 1981) or on concrete washed down with iodophor solution (1% v/v Iodel FS) between batches of plants (Tomlinson et al. 1981).

Direct treatment by the addition of pesticides to the growing medium of the individual cells of modules may offer a method of control for pests and diseases. Phytotoxicity, however, may be a problem particularly with insecticides where a change in the module system represented a reduction in the volume of growing mixture per cell but the amount of insecticide used remained unchanged (Thompson et al. 1982). Thus a change from 4.3 cm blocks (80 ml/cell) to cells (9-15 ml capacity) for brassica transplants precludes the addition of an approved insecticide at the correct rates to control cabbage root fly.

Fungicides are much less toxic when mixed with the growing medium for use in modules. Only transient growth checks occurred when pure calomel (about 0.5 g/l peat compost) was incorporated into 4.3 cm blocks to control clubroot. Calomel incorporations more so than those of thiophanate-methyl substantially increased yields of marketable brassicas (Ann et al. 1983) which were greater than those from bare root transplants treated with the same fungicide (Channon & Stewart 1984). In modular systems with smaller cells (13 ml volume/cell) control of clubroot and improved yields have been obtained with brassica transplants from Hassy trays treated with calomel (0.035 g to 0.05 g fungicide per cell) - (Ann, pers. comm.).

Tomlinson & Faithfull (1979a; 1980) prevented the development of lettuce big-vein disease in 4.3 cm block-raised lettuce by the addition of 0.025 g/block of Bavistin (50% carbendazim) to the peat before blocking. The product was toxic to zoospores of the vector fungus O. brassicae as were many surfactants including Agral, Cetrimide, Deciquam and Ethylan CPX (Tomlinson & Faithfull 1979b).

In commercial practice, lettuce in blocks containing incorporated Bavistin may be planted into the field or in recirculated nutrient solutions (NFT systems) in glasshouses. In NFT systems growers may also add Agral (20 ug/ml every 4 days) to the nutrient solution (Tomlinson & Faithfull 1979a; 1980).

Incorporation of the dicarboximide fungicides iprodione and vinclozolin at 1.20 g a.i./kg compost to blocks containing onions to prevent attacks by soil-borne Sclerotium cepivorum (white rot) reduced yield (iprodione) or were very phytotoxic (vinclozolin). Pre-planting drenches of these fungicides (20.5 g a.i./m^2 compost) to blocks gave increased yield and reduced white rot (Wafford 1984). Etridiazole, propamocarb, chlorothalonil and quintozene are incorporated in practice to control damping-off and wirestem fungi in blocking compost (Anon 1985).

Block incorporations and drenches were used by Crute (1978) to demonstrate that the acylanaline fungicides metalaxyl and furalaxyl could, by systemic action, protect young lettuce foliage from attack by Bremia lactucae (lettuce downy mildew). Block incorporations of metalaxyl (not an approved use) resulted in the production of up to 20% more brassica transplants but it also encouraged the development of resistance in Peronospora parasitica to the fungicide (Crute et al. 1985). Treatment of foliar pathogens in traditional transplant beds or in modular systems may be best achieved by the application of broad spectrum fungicides such as dichlofluanid.

PROSPECTS AND PROBLEMS

Seeds and transplants are the source materials of vegetable crops and as such are distributed widely. If these are infected then the resulting crops become diseased and severe losses may ensue.

In the past 20 years the development of selective eradicant fungicides and the facility to formulate them with one another and with protectant fungicides has broadened the range of fungicidal activity providing a wide spectrum of pesticides which can be used in a variety of ways to treat large quantities of vegetable seeds. The recent interest in the technique of film-coating appears to have considerable seed dosing and field drilling advantages over other seed treatment methods. In addition, it offers seed merchants the chance to produce a seed treatment mixture which he can identify by the addition of a dye, as his product and sell to his advantage. If subsequent biological and chemical tests confirm the effectiveness of this treatment, it may have application for many vegetable seed types, but for some, for example, beetroot (the existing thiram soak treatment) and celery (potential hormone/fungicide soak mixtures) (Gott et al. 1985) seed immersion treatments may be preferred for efficiency of disease control and improvement of emergence. There is no reason however why soaked seeds should not be film coated after treatment for ease of drilling or to incorporate an insecticide.

The development of identifiable seed treatments should encourage seed merchants to treat routinely more of their vegetable seeds. Too often treatment is only applied at the request of the grower. Seed treatments can ensure germination (controlling seed-borne organisms) and emergence (controlling soil-borne organisms) but usually they do not increase (with the exceptions already noted) the viability or vigour of seeds. Where disease control in modern transplant systems is a major concern it is imperative that disease tested and/or treated seeds of high vigour are sown to achieve the 95% establishment of healthy plants, necessary to make the system viable. Thereafter, the maintenance of their health status requires that the propagation area is disinfected and that the growing mixture is treated to kill potential pathogens. Problems, however, are likely to occur with the change to transplant modules containing larger numbers of cells of reduced individual volume particularly if pesticides are incorporated at effective dose rates in these smaller cells. Furthermore, the effectiveness of certain systemic fungicides against soil-borne fungi may be nullified by their selection of resistant isolates of foliar pathogens in the same crop.

The changes occurring in seed treatment and vegetable transplant technology should be supportive of each other with ideally protection against seed, soil and air-borne pests and diseases being achieved with a single treatment.

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THE PRODUCTION OF TOP AND SOFT FRUIT PLANTING MATERIAL FREE FROM BACTERIAL AND FUNGAL PATHOGENS

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ABSTRACT

Healthy planting material of fruit crops is produced by specialist growers of the Nuclear Stock Association. Measures are taken to exclude diseases of limited distribution or which become damaging when introduced with the planting material and for which control is difficult. The control of red core and other diseases of strawberry through Certification is limited by the sensitivity of detection. Chemical control in runner beds is discouraged because of masking. The recognition that increases of silver leaf and apple canker derive from mother plants has led to routine fungicidal controls and modified budding techniques. Alternative methods of producing planting material free from bacterial diseases include biological control of cherry crown gall and exclusion of bacterial canker of plum by micropropagation. Legislation and cultural practices restrict spread of fireblight. In future, more efficient production of disease-free planting material will be achieved by safeguarding the health of source plants, and by micropropagation.

INTRODUCTION

Vegetative propagation provides many pests and pathogens with opportunities for dispersal, either as contaminants or infections of planting material. The health of the planting material of fruit crops is a major determinant of productivity, and it was in recognition of this that nuclear stock schemes were set up for both top and soft fruit in the U.K. and elsewhere.

In theory, the elimination of all pathogens, viruses, bacteria and fungi from the nuclear source plant, followed by propagation under conditions of total isolation will provide completely healthy plants. In practice this is only achievable using micropropagation techniques. For fruit crops such techniques have yet to make a major contribution to the bulk of commercial planting material because of their high costs and continued concern over the production of 'off-types'. Also it is unnecessary to produce plants axenically as many of the pathogens, e.g. mildew, are of such frequency that reinfection will occur as soon as the plants are set out in the field. Instead, the current schemes seek to control by exclusion only those pathogens which do not establish quickly in the field unless introduced with the planting material. The number of bacteria and fungi recognised as belonging to this category has increased in recent years and it is likely that as micropropagation becomes less expensive this will be the preferred technique for their control.

Conventional propagation techniques, executed under glasshouse or field conditions, carry risk of infection no matter how good the isolation conditions appear to be. Consequently it is essential to have techniques which will detect infection before symptom expression. The greatest danger comes from cryptic infections which do not develop into field disease until after planting-out. It is important to recognise that these techniques, no matter how sensitive, are subject to sampling error and consequently cannot be used to prove the absence of the pathogen.

BACTERIAL DISEASES

Plant bacterial diseases are notoriously difficult to control, largely because of the lack of effective bactericides. Antibiotics, such as streptomycin, though giving good control, are prohibited in most European countries. Although a good case could be made for their use during propagation, which is unlikely to affect the ultimate consumer, there is the ever present problem of development of resistant strains. Copper, in various formulations, has been the chief means of combating bacterial diseases of fruit, but its use is limited by phytotoxicity. Resistant varieties may provide the long-term solution to many of these bacterial diseases but so far the fruit quality of such varieties has not been commercially acceptable. New approaches are, therefore, being developed against bacterial plant pathogens.

Crown gall, caused by Agrobacterium tumefaciens was, until a decade ago, an intractable problem. This soil-inhabiting pathogen enters the plant through wounds on the crown or root system inducing uncontrolled cell-proliferation and ultimately the formation of unsightly galls, rendering the plant unsaleable. The discovery by New & Kerr (1972) that strain 84 of the saprophytic A. radiobacter was antagonistic to the pathogen has revolutionised crown gall control. In Australia the incidence of the disease in peach nurseries has been reduced from a frequency of 80% to virtually nil and the high economic losses to nurserymen eliminated.

This biological control, the first for a bacterial plant disease, has been shown to be highly effective for crown gall on the cherry rootstock, Colt, at East Malling (Garrett & Fletcher, this volume). Strain 84 is now used in many countries for the production of stone fruits and roses. However, it is a preventive and not a curative treatment and furthermore, acts only against those strains sensitive to agrocin 84, i.e. the specific adenine nucleotide bacteriocin produced by strain 84. In general, the disease has not been controlled by strain 84 on apple rootstocks, cane-fruits or grapevines. For these hosts, gall-free material can only be produced by propagation from healthy stock in land free of the pathogen.

Bacterial canker of stone fruits, a problem in all areas where the crops are grown, is caused by Pseudomonas syringae pathovars syringae, morsprunorum and persicae. These pathogens are epiphytic on the leaves of their host where they sometimes give rise to leaf spots. The destructive phase of the disease is the cankering that develops in winter and spring, mainly from leaf scar infections on cherry and peach (pvs morsprunorum and persicae respectively) and trunk infection on plum (pv. morsprunorum) and to a considerably lesser extent, on all hosts in the U.K. by pv. syringae. The disease can be controlled on cherry and peach by an autumn spray schedule of Bordeaux mixture that eliminates

the epiphytic population available for leaf scar infection both in the nursery and in established orchards. There is no satisfactory control, as yet, for the disease on plum.

It is known that these pathogens are introduced into new orchards on the young trees from the nursery. Young trees become infested with bacteria from epiphytic populations of the pathogen on the mother trees through transfer on the buds or graftwood. In an attempt to produce healthy planting material, micropropagation methods have been used at East Malling where a pathogen-free orchard has been established remote from other orchards. Trees monitored over two seasons have remained free of P.s. pv. morsprunorum whereas in another orchard of similar age, established from traditional nursery material, two trees have already died from canker and the pathogen is widespread (Garrett & Fletcher, this volume).

Fireblight of apples and pears caused by Erwinia amylovora presents a special problem for nurserymen, for which the only available control measure is exclusion. Stringent regulations govern the movement or export of nursery trees to prevent spread of this pathogen. These prohibit the movement of even apparently healthy trees from nurseries in which any infection has been detected, and consequently the impact on trading can be very serious.

The risk of fireblight in the nursery can be reduced by some cultural practices. For example, the boundary hedges of agricultural holdings are frequently formed from fireblight-susceptible hawthorn. Removal of such hedges is obviously desirable but where this is not possible, trimming to prevent flowering is essential. Nurserymen can also seek to dissuade their neighbours from growing highly susceptible ornamentals, which for this reason are now being banned in specified areas of the Netherlands (Meijneke 1984).

FUNGAL DISEASES

Strawberry

Red core, caused by Phytophthora fragariae is perhaps the best known example in fruit of a fungal pathogen distributed with planting material. Following the first records in Scotland in the 1920s the pathogen has been distributed widely through the British Isles, mostly on strawberry runners. Further spread can only be prevented by certification procedures. In England and Wales the disease is notifiable under the Red Core Disease Order of Strawberry Plants (1957) which prohibits the sale and distribution of runners from land known to have been contaminated by the pathogen. Holdings on which more than 10% of the land is scheduled under the Order may not produce runners. As all but the highest levels of the nuclear stock scheme are field grown, these restrictions are clearly very important for preventing spread of the pathogen.

Efficient chemical control for red core is available (Montgomerie & Kennedy 1983). Because of widespread infestation in strawberry growing areas and the efficacy of such control measures, it has been argued that stringent precautions against red core during propagation can be relaxed. Such an argument does not take into account the risk of resistant strains developing. The use of fungicides during propagation raises matters of general principle applicable to all nuclear stock schemes. Whilst it is desirable to control non-scheduled pathogens, such as mildew, during

the propagation cycle it is highly undesirable to use fungicides which might mask infection by scheduled pathogens. This is especially true during the main bulking-up phases of propagation when a pathogen like P. fragariae could be disseminated over a wide area, albeit at low inoculum density.

Imported and newly introduced varieties of strawberry pose a particular risk of introducing red core into the nuclear stock scheme. Certification by inspection of plants for symptoms is quite unreliable. Indeed, much of the dissemination in Britain and Europe generally may have been through certified runners carrying infection at levels too low to be detected by field inspection. The introduction of a more sensitive test (Duncan 1980) in which root tip samples are baited with the highly susceptible Fragaria vesca may help to contain further spread. This test is now routine in Scotland for all certification grades and its use for foundation stocks in England is being considered.

Crown rot of strawberry, caused by a strain of Phytophthora cactorum, is a more recent problem than red core but resembles it in several respects. The pathogen was probably introduced to the U.K. from Continental Europe in the late 1960s and may have been unwittingly disseminated with certified stocks (Harris & Stickels 1981). Although field conditions in Britain are generally unfavourable to the pathogen, damage can be sustained in highly susceptible varieties and in protected crops. Detection before symptom expression is possible by incubating petiole samples in water for 24 h followed by microscopic examination, but the reliability of this method may only be 50%. This is now a routine procedure only in nuclear stock plants.

Strawberry wilt (V. dahliae) is another soil-borne disease where spread is controlled through Certification. This disease is notorious for symptomless infection and it is probably fortunate that damage is determined more by the infestation of the planting site than the planting material. Runner producers on wilt-prone sites are obliged to use expensive soil disinfestation by fumigation to meet certification requirements.

Black rot of strawberry, caused by Colletotrichum acutatum is a very recent example (Talboys et al. 1984) of a pathogen introduced from overseas with new varieties. Accidents of this nature would be much less likely to occur if the international exchange of plant material was through micropropagated material supplied in axenic culture.

Top fruit

The majority of fungal pathogens of apple, pear, plum and cherry are of such ubiquity that it has not hitherto been considered necessary to do other than control their associated diseases through fungicidal treatments during propagation. Recent evidence suggests that this approach may have to change with respect to Chondrostereum purpureum, causal agent of silver leaf in many hosts but particularly plum, and Nectria galligena, cause of European canker in apple and pear.

Following a recent increase in the incidence of silver leaf symptoms in certified material, a close examination was made of rootstock hedgerows and scion mother trees of plum. A relatively high incidence was recorded in some clones and varieties over a three-year period (Jeger

et al. 1984), although the symptoms fluctuated markedly within each season. By taking cuttings from rootstock hedgerows with or without obvious silvering, it was possible to demonstrate that the pathogen was transmitted by propagation. The wounds made to rootstock hedgerows and mother trees increase the likelihood of infection by C. purpureum. This, coupled with the uncertainty of symptom expression as a means of detecting infection, suggests that either a more sensitive method of detection needs to be developed or a routine prophylactic measure devised. The possibility of using serological methods to detect mycelium in wood chip samples has been examined but the polyclonal antisera produced were relatively inefficient (M.F. Clark, personal communication). The routine use of systemic fungicides seems more promising (D.J. Butt, personal communication) but more observations will be needed to ensure that such measures do not merely delay symptom expression.

There has also been a serious increase in the incidence of canker (Nectria galligena) in commercial plantations of apple in England in recent years, particularly in younger plantings. The problem was most noticeable in highly susceptible varieties such as Spartan but has also been observed in Bramley's Seedling, generally considered to be moderately resistant. Many infections undoubtedly occurred in situ through leaf scars etc. However, a previously undescribed method of spread within trees was also observed, namely movement within the vascular system leading to virtually systemic infection. The implications of this observation have yet to be fully explored, however the risk of transmission during propagation in a manner comparable to C. purpureum must now be seriously considered. Bennett & Vaughan (1971) had already demonstrated that conidia of N. galligena could be carried on buds from infected mother trees and recommended that benomyl should be used routinely to suppress spore production on such trees. It was also shown (Bennett & Hulland 1973) that dipping budwood in dodine or other fungicides reduced the incidence of canker and that infections were less likely with chip-budding than T-budding. In general both of these measures are now standard practice, but would only control infection by contaminating conidia.

FUTURE PROSPECTS

Over the years there has been a steady progression towards greater isolation and containment of fruit plants within the nuclear stock schemes, especially at the higher levels. Even top fruit mother plants are now held in protection under gauze to reduce the chance of infection. Growing media are routinely sterilised before use and strawberries, for example, are now raised in hydroponic culture to reduce the likelihood of infection by nematodes. Micropropagation will undoubtedly facilitate the production of disease-free plants. There has also been an increase in the use of soil sterilants such as chloropicrin and formaldehyde to combat poor growth in the nursery. Sewell & Roberts (1984) have demonstrated the importance of VA mycorrhizae to the growth of apple and thus highlighted the danger that planting material could become too 'clean'. Total exclusion of harmful bacteria and fungi is also likely to lead to the loss of beneficial organisms. Consequently there will be a possible requirement to add microbial inoculants to planting material.

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1986 BCPC MONO. No. 33 SYMPOSIUM ON HEALTHY PLANTING MATERIAL

POTATOES - THE IMPORTANCE OF PRODUCING DISEASE-FREE PLANTING MATERIAL

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ABSTRACT

Many fungal and bacterial diseases affect potato seed tubers and most cause yield losses and are a source of infection in succeeding crops. Healthy planting material can be produced by vegetative propagation but improved husbandry and crop protection chemicals will be required to maintain health during multiplication so that seed and ware producers benefit from less disease.

INTRODUCTION

The potato is notorious among major arable crops in attracting many diseases and disorders. Although some are found wherever the crop is grown, the occurrence of others is restricted by geography, climate or by stringent regulations. Not only are the diseases caused by different groups of agents (viruses, bacteria, fungi) necessitating different control strategies, but many are damaging throughout the 18-month crop cycle from receipt of seed tubers to disposal of stored ware tubers. That the method of propagation by vegetative tubers contributes to the problem of disease becomes evident when it is appreciated that the majority of agents can be carried on or in the seed tuber, permitting dispersal in both space and time.

Fungi and bacteria cause damage to seed tubers and to growing and stored crops; many diseases are present in most crops each year but they can be capricious and occasionally cause large losses, perhaps without warning. In all seasons late blight and the blackleg/soft rot complex pose a threat to the health of crops with the potential to cause catastrophes. The importance and prevalence of other diseases has varied in past decades, for example dry rot was prevalent in the 1940s, skin spot in the 1960s, gangrene in the 1970s and the canker form of powdery scab in the early 1980s.

PREVALENCE AND EFFECTS OF DISEASES

During the early 1960s when damage to seed tubers by skin spot was common, considerable interest developed in the occurrence of diseases carried on seed, and, in 1962, we started an annual examination of seed samples from farms in England and Wales in an attempt to assess the prevalence of diseases in the national crop. The information collected over 14 years indicated that some diseases were always found on the majority of tubers whereas others affected fewer tubers (Hide 1981); their occurrence also differed between years, cultivars and areas of production. The mean annual incidence of most diseases differed by a factor ranging from 3 to 5 (Table 1) but the range of blight infection was larger, indicating its greater dependence on weather conditions during crop growth. Perhaps even more striking was the difference in disease incidence between seed stocks (Table 1). Whether the year was considered 'good' or 'bad' for a disease, the worst affected stocks had similar amounts of disease. Conversely, there were always some stocks with little or no disease despite a high mean incidence.

In a survey of commercial seed stocks in Scotland in 1966-68, most tubers of all cultivars examined were contaminated with Erwinia carotovora, whether they were taken from crops that had or had not shown blackleg in the

TABLE 1

Lowest and highest mean incidence of diseases on seed during 1963-76 and the range from different seed stocks

Disease	Year	Mean % tubers affected	Range of infection (% tubers) in different seed stocks
Skin spot	1973	25	0 - 86
(Polyscytalum pustulans)	1963	78	8 - 100
Gangrene	1973	3	0 - 32
(Phoma exigua)	1975	12	0 - 50
Black scurf	1963	9	0 - 60
(Rhizoctonia solani)	1976	42	4 - 90
Silver scurf *	1966	19	0 - 82
(Helminthosporium solani)	1972	60	1 - 96
Common scab	1964	13	0 - 78
(Streptomyces scabies)	1970	34	0 - 100
Powdery scab	1963	8	0 - 68
(Spongospora subterranea)	1971	26	0 - 84
Blight	1974	0.3	0 - 6
(Phytophthora infestans)	1967	2.8	0 - 30

* assessed as Helminthosporium conidiophores on tuber eye plugs

field. About 80% of the isolates were identified as subsp. atroseptica, the cause of blackleg (Pérombelon 1972).

Although such data are interesting, the incidence of disease does not equate with importance. For example, 2% tubers with late blight can be potentially more important than 25% with black scurf or 60% with skin spot. Seed tubers with blight may be the cause of epidemics affecting many plants in the crop, but with other diseases effects on growth and yield are mostly limited to the seed tubers themselves and the plants produced by them. Blackleg, gangrene, stem canker and skin spot cause gaps in crops or modify plant growth and tuber production but, provided diseased plants are randomly distributed amongst vigorous neighbours, inter- and intra-plant compensatory growth ensures a reasonable yield if the crops are grown to maturity; larger penalties may result when plants are harvested early. As tuber numbers and size are also affected both by disease and by compensatory growth, so the saleable yield may be altered. Effects on yield of other seed tuber diseases such as common scab and silver scurf are transient and mostly confined to the first weeks of growth.

DISEASE TRANSMISSION

Planting diseased seed also prejudices the health of the crop. All the diseases given in Table 1 as well as blackleg/soft rot, can be transmitted by the seed tuber and, except for common scab, this is probably the major source of inoculum. After planting, pathogens grow vegetatively (Rhizoctonia) or sporulate on the seed tuber (Polyscytalum, Phoma, Fusarium, Helminthosporium) or rot it (Erwinia, Phoma, Fusarium); root systems are

attacked (Rhizoctonia, Polyscytalum) and daughter tubers become infected or contaminated with inoculum. However, relationships between amounts of disease on seed tubers and on daughter tubers are usually poor and there may be several reasons for this. It is likely that many seed tubers apparently free from disease do bear cryptic or latent infections or adhering inoculum. For example, seed tubers apparently without gangrene can give as much disease in a stored crop as badly rotted seed (Adams et al. 1980). Similar transmission from latent inoculum can occur with Spongospora, Fusarium and especially Erwinia. By contrast, seed tubers bearing small lesions of silver scurf often give more disease in store than those bearing extensive lesions that have less ability to produce conidia. With Erwinia, the amount of inoculum on or round seed tubers is variable and depends on soil conditions. Consequently, freedom from disease is not synonymous with freedom from inoculum or infection, and it is therefore unlikely that roguing out diseased seed tubers (negative selection) would improve crop health quickly.

Transmission of infection from seed tubers is affected by environmental factors including soil conditions and type. Dry conditions after planting encourage the development of stem canker and so provide ample inoculum for tuber infection. Moist soil increases rotting of seed by Erwinia and encourages infection of stem bases by Polyscytalum and of skin spot later in storage. Irrigation increases black dot (Colletotrichum) on tubers but decreases silver scurf.

Occurrence of diseases is also influenced by the site where the crop is grown, and is undoubtedly affected by weather and soil type. In recent experiments, large differences in infection were detected between sites two months after planting and were often greater than differences attributable to seed tuber-borne disease. Such factors cause difficulties in forecasting the likely amount of disease in store, and in different seasons, crops with similar amounts of infection or inoculum at harvest and stored under identical conditions have developed widely differing amounts of disease during storage (Table 2). Alternatively, similar amounts of disease after storage developed from crops with widely differing amounts of infection assessed at harvest. With these apparent anomalies, it may be pertinent to question the methods of assessment and also whether tubers carry not only inoculum or infection, but also factors as yet unknown that determine susceptibility to disease development during storage.

Although the contribution of soil inoculum should not be overlooked, the main source of inoculum is the seed tuber and we are fortunate that most of the important diseases are primarily seed tuber-borne; fortunate because inoculum is localised and, with varying facility, is amenable to control. Certainly the problems of controlling soft rot and gangrene would be much greater if most disease originated from inoculum in the soil.

CONTROLLING TUBER-BORNE DISEASES

Because the visual selection of apparently healthy seed tubers was unlikely to contribute to the improvement of crop health, alternative methods were sought. One technique is to use stem cuttings; aerial shoots from virus free plants are excised, rooted in sterilised compost and grown in fields long free from potatoes to produce healthy nuclear stocks of tubers. These are then multiplied for several years under farm conditions as virus-tested stem cutting seed (VTSC). More recently, smaller stem segments, which may decrease the chance of disease carry-over, are grown initially under aseptic conditions (micropropagation); this method is now used in potato nuclear

TABLE 2

Assessments of infection (Polyscytalum, Helminthosporium) or inoculum (Phoma, Erwinia)at harvest and amount of disease after storage

Disease	Year	Harvest	Post-storage
Skin spot	1975	23	13
	1974	23	65
Silver scurf	1973	13	33
	1974	13	84
Gangrene	1975	6	6
	1972	6	10
Soft rot	1971	2	0.1
	1972	28	0.1

stock production in several countries. However, when healthy stocks are incorporated into existing commercial multiplication programmes, it is to be expected that they will not remain healthy for long, and during the first years when VTSC progenies were becoming available to ware growers (as Foundation Stocks), it was difficult to detect any improvement in health compared to stocks multiplied by traditional tuber selection methods (Hide 1981). Similarly, in 1973 and 1974, VTSC stocks in their fifth year of multiplication were already extensively contaminated with Erwinia bacteria (Pérombelon et al. 1980).

Investigations stimulated by this apparent breakdown in health revealed alternative sources of contamination. Erwinia bacteria have been detected in wind generated aerosols (Graham et al. 1977) and on insects (Harrison et al. 1977); fungal spores of Phoma and Polyscytalum were found in the soil and dust from potato stores (Carnegie et al. 1978) and Phoma spores were caught in field-located traps (Carnegie 1984). These hitherto minor sources of inoculum have now assumed greater importance because the major source has been avoided. During multiplication of stocks, small amounts of acquired inoculum increase on growing plants and in store, and the difficulty of maintaining standards of hygiene increase the larger the crops become (Hide 1978). One approach to avoiding some of the increase in disease is to decrease the number of years that stocks are multiplied before disposal, and here micropropagation techniques are important. Another, but as yet unproven method would be the use of true seed; using this technique it should be possible to produce much healthier crops, although plant pathologists would recognise that if many of the currently important pathogens are removed, there are likely to be others to fill the vacated ecological niches.

MAINTAINING HEALTH

For the immediate future, much of our effort must aim to prolong the health of nuclear stocks. Chemical tuber treatments are available and both 2-aminobutane and thiabendazole are effective against a range of pathogens and have gained commercial acceptance. Although 2-aminobutane used on diseased seed has not improved the health of progeny tubers, it is probable

that, like organo-mercury compounds in the 1960s, it would maintain health when amounts of inoculum are small. But benzimidazoles, including thiabendazole, as well as other fungicides have a longer lasting benefit and, after application to diseased seed, decrease the amount of infection at harvest and prevent disease development in store (Cayley et al. 1983); effects of a single treatment can last for up to 18 months. The success with these materials seems to lie in their persistence on the seed tuber throughout crop growth preventing escape of inoculum. Consequently, the materials must be applied to the whole tuber surface for there is little lateral or internal movement after deposition. However, it is disappointing that commercially treated seed sometimes carries negligible fungicide residues or at best, 25% of the applied dose; furthermore, it may be unevenly distributed over the tuber surface. The use of electrostatically charged sprays should improve deposition and with it the efficiency of the treatment so that it might be possible to decrease the amount of chemical used.

There is currently less optimism for the control of blackleg and gangrene on progeny tubers by chemical seed treatments. Blackleg bacteria, located in lenticels or the stolon scars are safe from toxic materials deposited on the tuber surface; they rot the seed and migrate into stems, and materials that have performed well in vitro have failed to control the disease. It is possible that treatment before the lenticels have become sealed would be more effective but this would probably require immediate treatment after early harvest. Internal rotting and migration of pycnidiospores into stems might well be the explanation for the failure of fungicide seed treatment to control gangrene in daughter tubers.

BENEFITS OF HEALTH

Seed stocks derived from stem cuttings, multiplied in Scotland after annual treatment with fungicides and grown at Rothamsted for one year (healthier seed), were used in experiments during 1965-76. As it was known that the amounts of disease in commercial seed stocks differ widely, it would not have been easy to choose 'average' stocks for comparison; indeed we could have been tempted to select bad stocks to exaggerate the benefits of health! Instead, samples of seed from up co 20 stocks of 7 cultivars were used, and from 1965-75 healthier seed averaged 7% more total yield at harvest than commercial stocks, and considerably more in 1976 (Table 3). These annual differences were to be expected and probably resulted from differing amounts of disease on both the commercial and also on the healthier stocks which had acquired small amounts of infection during 5 or 6 years' multiplication.

Yields of ware-sized tubers (> 44 mm) were not increased to the same extent as total yield because one feature of healthy seed is that it produces plants with more stems and smaller tubers. In some years plants from healthy King Edward seed produced twice as many tubers and less weight of ware than commercial seed, and this perhaps may be one reason for the demise of this cultivar. Other cultivars have been less affected (Table 4), but the propensity for producing more even-sized small tubers is of benefit to seed producers.

The use of healthier seed also results in less disease developing during storage, so that not only are harvested yields increased, but also there is less loss of saleable crop after storage. The extent of this benefit is not easy to measure because tuber quality is subjective. In any season soft rot, gangrene and dry rot are unacceptable in a ware sample, but the importance of superficial blemishes is affected by market pressures. For example, in

TABLE 3

Comparison of total yields from commercial and healthier seed stocks

Year	Number of seed stocks		Mean total yield (t ha^{-1})		Benefit from health (%)
	Commercial	Healthier	Commercial	Healthier	
1965	1	1	77.9	85.3	9
1967	1	1	59.7	62.1	4
1968	3	3	45.4	47.9	6
1969	4	4	42.5	46.0	8
1970	4	4	42.2	44.3	5
1971	4	4	47.7	53.6	12
1972*	46	8	42.1	47.1	12
1973*	26	8	46.8	50.0	7
1974	23	10	54.6	57.2	5
1975*	20	10	23.9	24.6	3
1976*	6	5	29.2	36.6	25

* included irrigation treatments

1975 when yields were low and supplies scarce, crops that in some years would have been rejected were saleable. By contrast in 1985, buyers were willing to pay a substantial premium for potatoes free from silver scurf, a minor blemishing disease.

TABLE 4

Comparison of total and ware yields (t ha^{-1}) from commercial (means over 20 stocks) and healthier seed of two cultivars, 1972

		Commercial	Healthier
King Edward	Total	42	44.8
	Ware	28.6	27.7
	% ware	68	62
Pentland Crown	Total	39.8	42.2
	Ware	34.3	36.0
	% ware	86	85

CONCLUSIONS

Seed tuber-borne diseases are important as a cause of yield loss and as a source of infection in succeeding crops. Methods are available for producing healthy planting material but it is imperative to prosecute health throughout seed multiplication and so improve the product available to ware producers; also ways need to be found to obtain the maximum benefits from healthy seed. This will involve not only pathologists, chemists and those concerned with the agronomy of the crop, but also plant breeders who have a

significant contribution to make in producing cultivars less susceptible to disease, thereby decreasing reservoirs of inoculum. When healthy crops are the norm, management and storage conditions can be imposed that best befit the crop rather than adopting a compromise procedure to minimise disease incidence.

Seed and ware producers are not the only losers from potato diseases. Loss of yield and quality affects merchants, exporters, processors and consumers who all bear some of the costs of suffering or avoiding diseases, whether from lower yields, expenditure on classification schemes, plant breeding and crop protection chemicals. Some years ago the Potato Marketing Board indicated that less than two thirds of the crop produced reaches market because of mechanical damage and disease; a poor record for an industry that is considered by some to be among the most efficient.

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PROTECTED CROPS: THE PRODUCTION OF HEALTHY BULBS AND ORNAMENTALS

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ABSTRACT

The need for adequate profit margins in the face of increasing costs has led growers of protected ornamentals towards specialist monocropping. This, in turn, increased the risk of economic losses through disease which has been limited by the use of genetically resistant plants, screening plants for health and good crop husbandry. Three crops (carnations, chrysanthemums and bulbs) are considered in detail.

INTRODUCTION

Bulbs and ornamentals as protected crops conjure up different images to different people and this area of the horticultural industry has become and continues to become more and more specialised. Twenty or thirty years ago most growers would have had mixtures of crops of roses, carnations, natural season chrysanthemums, tomatoes or lettuce. A common rotation was spring lettuce, summer tomatoes followed by natural flowering chrysanthemums. At some time during this period, usually before the tomato crop, the soil would be steamed using Hoddesdon pipes and a mobile boiler supplied by a contractor.

The beginnings of specialisation commenced many years earlier when, for example, cucumbers with their special requirements were grown in houses unsuitable for other crops whilst in the Spalding area, daffodil and tulip bulbs were prepared for forcing as an early spring crop before tomatoes.

There are six main divisions of the protected ornamental trade which in 1983 was valued at £79.1M (Table 1). Official figures are not available for these divisions in earlier years but the impressions are that the home produced share of the market for carnations, roses and chrysanthemums is tending to decline whilst bedding plants, bulb flowers and miscellaneous pot plants are increasing. The loss of a market share is usually attributed to cheaper production costs, i.e. carnations from Colombia and Israel, but the reasons are more complex.

TABLE 1
Value (£M) of protected ornamentals in 1983

Bedding plants	19.6	
Bulb flowers	15.5	
Carnations	6.1	
Chrysanthemums (cut)	9.9)	
Chrysanthemums (pot)	5.1)	15.0
Other pot plants	21.4	
Roses	1.5	
	79.1	

Horticulturists have never had the protection against competition common in other sectors of agriculture and consequently they are more responsive to changing ideas or markets. The two principal ones in the past three decades have been the need for more efficient management and the adoption of new techniques and materials, a combination which is often so intertwined as to be inseparable. In this climate of competition, quality has been and still is as important as productivity.

In the changes that have taken place the most far reaching has been the move from mixed cropping to monocultures. Both flower and vegetable growing became the province of the specialised grower, and as labour costs increased, this was also coupled with larger holdings.

Once the concept of single cropping was widely accepted, the pressure for better yielding and better quality products was felt by both producers and consumers. The old inadequately yielding carnation varieties were swept away by the Sim types; all the year round flowering chrysanthemums (AYR) became available and techniques to produce earlier and earlier bulb flowers were developed.

The initial development of AYR chrysanthemum and carnation cutting production by specialist propagators was very successful. However, with hindsight, one can see that this phase was the lull before the storm in pathological terms as losses from disease soon reached proportions which threatened the future of the industry. The market for cuttings depended not simply on healthy cuttings but customer confidence. Customer confidence was based upon, amongst other things, a very low incidence of disease: not simply to a level that was acceptable in economic terms, but to a level tantamount to no disease at all. The flower producer demanded the same quality of the propagator as his market salesman.

Production of flowers from ornamental bulbs was very different. The techniques used were not new and both yields and quality seemed to be satisfactory. However, this view was shown to be false, not by the growers themselves, but by scientists who demonstrated almost complete infection of stocks with virus diseases.

Both growers and scientists working with each of these crops produced different solutions to the problems of plant health, which are best seen by charting the progress of each of the three crops over the past three decades.

Chrysanthemums

AYR chrysanthemum growing was introduced into the country from the United States of America in the early 1960s. The essential difference between AYR cultivars and the traditional natural season cultivars was that the former were photoresponsive whilst the latter were thermoresponsive. Growers who took up the new system found themselves in a horticultural strait-jacket. In the past, blooms were cut over a period of a week or more, largely because temperature gradients in the glasshouses caused differences in maturity. This was not unacceptable to the grower who, in effect, averaged his market returns instead of risking either high or low prices. Now their houses had to be heated uniformly and much lighting and shading equipment installed.

Only when all this was done and operating satisfactorily did the true uniformity of the crop become apparent; now large areas could and had to be cropped within a few days. One result of this was that poor quality plants, previously unnoticed, became glaringly obvious.

The two most obvious symptoms seen were shortened, early flowering plants and plants bearing distorted flowers. The former symptom was caused by infection with chrysanthemum stunt virus (CSV) which was in sap transmitted on the fingers of the people taking cuttings (Hollings 1959). The second was caused by the aphid-borne chrysanthemum aspermy virus (CAV). Controlling aphids was relatively easy but to control the incidence of stunt virus was to screen plants for freedom from infection. This could only be done by grafting onto an indicator plant (chrysanthemum cv Mistletoe) and waiting for up to six months for symptoms to appear. The testing for aspermy virus was relatively short, a matter of weeks, and involved inoculating tobacco plants with sap from the test cultivar.

It seemed prudent, because of the long delay in the stunt test, to check as many aspects of health as possible, and each cultivar was passed through a series of tests. The first was choosing cuttings from plants selected for flower quality and culturing them to show freedom from Verticillium alboatrum and V. dahliae both of which cause wilt. In practice it was found that a group of cultivars, the Jetfires, were very susceptible and when these were deleted from stockholdings the wilt problem became negligible. The cuttings were cool-stored during the seven days of the culture test. No attempt was made to identify bacterial or fungal growth contained in any culture tubes. Only cuttings associated with clear tubes were rooted and proceeded to the next test. In this, the rooted cutting was approach-grafted with a rooted cv Mistletoe cutting and both planted in a small isolated area of sterilised compost. After ten days, the stem of the Mistletoe cutting was severed below the graft so that it was wholly maintained by the test plant. During the next few months, cuttings of the test plant were taken, rooted in isolation and replanted in the isolated area. Additionally, the test for aspermy virus was done during this period. At the end of the test period, a small number of virus tested cuttings were then released for large scale commercial production. The tests themselves were relatively simple; the skill lay in testing the right number of plants at the optimum time of the year to provide sufficient cuttings for multiplication to meet the projected sales.

Although these techniques and the use of other indicator plants were designed to sift out plants with CSV and CAV, they also removed chrysanthemum virus B from the stocks. It took several years for the industry to achieve the desired level of health but during routine testing it was found that cv Fred Shoesmith was to all intents and purposes symptomless when infected with CSV and was probably the main reservoir of infection. A similar finding occurred with cvs White and Yellow Indianapolis No 3 for CAV (Knapman, personal communication). These individual tests have been known for many years. For example, the culturing test to detect wilt in carnations has been known for more than eighty years (Mangin 1899) and the CSV grafting test since 1949 (Brierley & Smith 1949).

It is assembling these tests into the most efficient sequence that is important in commercial horticulture. The American work on the topic has been reviewed recently (Raju & Olson 1985).

Much effort has been made in recent years toward rapid tests for both fungal and virus diseases and indirect enzyme linked immunoabsorbent assays seem promising.

However, just as the industry was accepting the role of specialist propagators, two further diseases became prevalent. The first, rayblight, long known in the United States of America as a disease of flowers, caused by Mycosphaerella ligulicola became rife as a stem and root disease. Work by Chesters & Blakeman (1956) showed that the fungus was able to survive in the typically wet conditions at the base of propagating beds. As soon as hygiene conditions were improved, the importance of the disease diminished. The second disease, Phoma root rot, was discovered in the UK in curious conditions. 'Natural season growers', who usually produced a range of blooms from October until December, began their sequence with cultivars grown out of doors, one commonly-used group being the Mayford Supremes. When, in order to increase their range of colour and form they planted AYR cultivars, some, such as Portrait and Criterion, either died or developed poorly (Kemp 1958, Hawkins 1968). Several hundred AYR cultivars were screened for resistance but few were found; one was Heyday. Piecing the story together, it became apparent that Phoma chrysanthemi was commonplace in outdoor beds and standing grounds and over a period of many years only those natural season cultivars that were tolerant had remained in widespread use. The parentage of the AYR cultivars was completely different and they had never been challenged by P. chrysanthemi. It had been common practice to steam glasshouse soils before the tomato crop which eliminated P. chrysanthemi from these soils.

Carnations

For many years the development of carnations as a profitable glasshouse crop was prevented because of their narrow flowering season. The main demand for carnations was and is for white flowers for Easter weddings but their natural propensity was to produce a flush of flowers later in June. Once the Sim type became widely available and it was shown that sequential planting and stopping dates coupled with autumn dawn to dusk lighting would extend the flowering period, the area planted might have increased (Bunt et als 1981; Bunt & Powell 1982). By 1974 it reached 70 ha but this has since declined to about 40 ha for several complex and interacting reasons, of which disease control and cheap imports are the most important.

Three decades ago, the principal pathogens were wilt (V. cinerescens), Septoria leaf spot and rust (Uromyces dianthi). However, most diseases declined in importance once specialist propagators appeared on the scene. The only exception was V. cinerescens but with routine testing of cuttings (Ebben 1962), routine steaming of soil and raised growing beds, this too declined. A similar system to that used with chrysanthemums was adopted by propagators. The first test removed cuttings with fungal infections whilst plants with virus infections were identified by a series of inoculations on to Gomphrena, Chenopodium and Nicotiana and a graft test on carnation cv Joker. Later, meristem tip culturing replaced the initial test.

Fusarium oxysporum f.sp. dianthi a disease known for many years (Wakefield & Bisby 1941 and Moore 1959) but described as being less important than V. cinerescens suddenly, in the space of a few months caused wilting in a large number of nurseries. The new aggressive strain had been imported on cuttings from South Africa and its rapid widespread dispersal is an example of the risks inherent in having few specialist propagators.

Fungi such as species of Fusarium that produce chlamydospores are not easy to control and the level of control achieved was never satisfactory. Many approaches were tried: soil sterilisation with chemicals as well as steam, spot treatment using fungicidal drenches, but to no avail. Slowly, spray carnations, which were wilt-resistant, replaced Sims but during this period when home production faltered, imports began to arrive, first from Israel and then Colombia. Breeding resistance into the Sims had begun in 1978 at the John Innes Institute (Arthur et al 1983) using the spray carnations, but unfortunately the proportion of home-produced flowers fell rapidly and the work, which contained promising hybrids, was discontinued.

Ornamental bulbs

Although the pattern of trade is changing from a simple sequence of forced tulips, trumpet daffodils and iris to include lilies and miniature daffodils in pots, the need for quality production remains. It is quality, in the sense of good health, that is governed more in the season preceding forcing than within the house during forcing that matters.

Whereas healthy planting material of chrysanthemums and carnations was relatively easy to find or produce, ornamental bulbs presented problems insoluble even by the most technically-minded grower. With very few exceptions most of the main cultivars used for forcing and flower production of narcissus, lily and iris were infected with several viruses. These bulbs were also prone to fungal infections which either reduced the numbers of bulbs lifted and/or the numbers of flowering-sized bulbs.

The relationship between numbers of bulbs planted and the proportion of flowering-sized bulbs lifted is very important. Diseases which reduce leaf area, such as leaf scorch of narcissus (Stagonospora curtisii) and tulip fire (Botrytis tulipae), reduce the sizes of bulbs rather than total number (in very severe attacks total numbers will be reduced). The commercial effect is that the proportion of saleable stock is smaller. Apart from the obvious loss of revenue the grower is left with a greater proportion of small bulbs to replant. Iris bulbs have to be a certain size before a flower is initiated, otherwise a condition known as 'drieblad' (plants with three leaves and no flower) occurs. Leaf diseases caused by ink disease (Drechslera iridis) or leaf spot (Mycosphaerella iridis) which cause early senescence are particularly damaging in this respect.

Other pathogens attack bulbs causing a numerical loss. Among these are Fusarium rot of tulips (F. oxysporum f.sp. tulipae) and basal rot of narcissus (F. oxysporum f.sp. narcissi).

Recently, it has been shown that the latter also causes a saprophytic rot of the flower stalk. To pathologists the former disease is of considerable interest because some, but not all, isolates of the forma speciales produce ethylene (Price 1975a). Tulip flowers in their early stages of development are very sensitive to this gas and damage can occur to bulbs whilst in store or even after planting. Thus, a small number of infected bulbs can damage a considerable number of healthy ones (de Munk 1971, de Munk & de Rooy 1971). It was also discovered, in epidemiological studies, that the outermost scale, whilst still fleshy, contains a fungicidal compound, tulipalin: later as the scale becomes membranous and the concentration of tulipalin diminishes, bulb susceptibility increases (Bergman 1966, Beijersbergen & Lemmers 1972). Although good control can be obtained using fungicidal dips, the most important factor is lifting the bulbs before they become susceptible.

Narcissus, unlike tulips, are usually grown on a two-year cycle. The most important cultivar is Golden Harvest. This is forced into early flowering and can produce as many as 35000 flowers/tonne. Together with Fortune and Carlton it forms the mainstay of the forced daffodil flower trade. Unfortunately, Golden Harvest is susceptible to basal rot and much research work has gone towards controlling the disease. Although the primary reservoir of the pathogen is in the soil, the important source of infection is within a stock. This danger comes from two sources: rotting bulbs and Fusarium carried on healthy bulbs (Price 1979 b & c). Basal rot was first described in 1886 but until fifty years ago was invariably confused with stem and bulb eelworm infestation (Ditylenchus dipsaci). When experimental work treating bulbs with hot water (HWT) controlled this (Westerdijk 1917) the full extent of fungal infection was revealed (bulbs were dipped for 3 hours at 44.4°C). Early work with mercurial fungicides added to HWT were very effective especially when the crop was grown annually (Gregory 1932, Hawker 1940, 1944). However, doubts about the use of mercurial fungicides led to greater use of formaldehyde. Over a period of many years, work at Kirton and Rosewarne Experimental Horticulture Stations and the Glasshouse Crops Research Institute showed that early lifting and early HWT coupled with annual planting for several seasons gave good control of the disease (Price & Briggs 1976).

Unfortunately growers were reluctant to change to annual cropping (standard practice in the Netherlands) and research efforts therefore concentrated on improvements to HWT. Formaldehyde is very effective in controlling Fusarium but some chlamydospores survive three hours' exposure in either cold or hot formaldehyde solution (C.A. Linfield, personal communication). Dipping bulbs in cold formaldehyde within forty-eight hours after lifting gave good disease control, whereas a longer gap between lifting and treatment was of little value. Linfield believes the explanation for this is that the longer the delay before treatment the greater the number of chlamydospores formed. Formaldehyde is volatile at ambient temperatures and has therefore, no residual effect. The efficacy of HWT plus formaldehyde can probably be improved by adding a fungicide with a residual effect to control the surviving chlamydospores. There is promise of success from this approach but several seasons' results are needed for confirmation (Linfield, personal communication).

The industry is obtaining temporary relief by using more Group 2 cultivars: these are large cupped daffodils, some of which are markedly but not completely resistant. Nonetheless, the long term answer has to be genetic resistance. Susceptibility has been associated with the parents of Group 1 trumpet daffodils with white cups such as Mme de Graaf (Fry 1968). Linfield (1985) suggested that susceptibility is derived from Narcissus pseudonarcissus and its sub-species. Certainly species such as N. bulbocodium, N. canaliculatus, N. henriquesii and N. odorus rugulosus are resistant and should provide a good source of material. Linfield & Price (1985) have recently devised a rapid screening method for resistance and a breeding programme began at the Glasshouse Crops Research Institute in 1984.

The provision of healthy ornamental plants in commerce seems to follow a set pattern: all plants need a consumer attraction but preferred plants must also have a minimal disease risk. Examples of these are Alstroemerias and Poinsettias. Then there are those plants which have pathological problems but with which the grower can cope, possibly with assistance from the scientist: chrysanthemums and pelargoniums exemplify this group. Lastly, there are those plants which present very difficult problems often because of the total infection with one or more virus diseases or extreme susceptibility to disease (usually wilt diseases): lilies, iris and daffodils fit in the first category, perpetual carnations in the latter.

Although the title of the paper implies the provision of plants free from fungal disease, commercial interests are rarely so well defined. The pathology of a crop, or inversely, the health of a crop embraces bacteria and viruses as well. The effective plant pathologist in commerce has to be a jack-of-all-trades: being as competent in screening for viruses as for fungi. Such scientists are few and far between and consequently, are well valued by industry.

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