4.
Pests

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REDUCING THE RISKS FROM NEMATODE PESTS OF VEGETATIVELY PROPAGATED CROPS ENTERED FOR CERTIFICATION

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ABSTRACT

Following the production of virus-tested, pest-free nuclear stocks, it is essential to minimise the risk of nematode infestation and nematode-transmitted virus infection during subsequent propagation. Measures to reduce the risks are taken at three stages. Pre-planting measures comprise soil sampling of sites, the use of loamless or sterilised composts for small-scale propagation and the avoidance of sites previously cropped with alternative hosts of particular nematodes. These measures are supplemented by inspection of the growing crop for symptoms of nematode infestation and virus infection, and post harvest inspection of bulbs and tubers for nematode infestation. The limitations of sampling to detect nematodes or the symptoms they produce in the crop are discussed. Despite the limitations of individual safeguards, combinations of measures can reduce risks to insignificant proportions.

INTRODUCTION

Some nematode species cause direct damage to crops by invading and feeding within plant root or shoot tissues. Others cause damage by feeding externally on roots or within leaf buds whereas other root ectoparasites are primarily important as virus vectors. The nematode pests which are of greatest importance in MAFF Certification Schemes for vegetatively propagated crops are shown in Table 1 with the crops which they attack. Research institutes and university departments produce virus-tested and pest-free nuclear stocks of these crops. This paper considers the measures which can be taken to reduce the risk of nematode infestation and nematode-transmitted virus infection of the crops during subsequent propagation cycles. Measures can be taken at three stages (Table 1):

1) Pre-planting : a) soil sampling of the sites to be used, or b) the use of loamless or sterilised composts for small-scale propagation, or c) the avoidance of sites previously cropped with alternative hosts of the nematode.

2) Growing crop: inspection for symptoms of nematode infestation and virus infection.

3) Post-harvest: inspection of bulbs and tubers for nematode infestation.

For some of the pests listed in Table 1, pre-planting measures are backed up by growing crop inspection. For other pests, pre-planting measures, particularly soil sampling, are inappropriate and reliance has to be placed on growing crop inspection and/or post-harvest inspection.

TABLE 1
Measures taken to reduce the risks from nematode pests of vegetatively propagated crops entered for certification

		Time of inspection and measures taken		
CROP	PEST	PRE-PLANTING	GROWING CROP	POST-HARVEST
SEED POTATO	POTATO CYST NEMATODES(PCN) *Globodera rostochiensis* *G. pallida*	Sites soil-sampled to detect PCN	Roots examined if PCN suspected but fields with population densities sufficient to produce crop symptoms would mostly be eliminated by soil sampling	Restriction on amount of 'dirt or other extraneous matter' which could harbour PCN cysts
	POTATO TUBER NEMATODE *Ditylenchus destructor*			Tubers inspected for nematode infestation
IRIS	POTATO TUBER NEMATODE			Bulbs inspected for nematode infestation
NARCISSUS	STEM NEMATODE *Ditylenchus dipsaci*		Plants inspected for symptoms of nematode attack	Bulbs inspected for nematode infestation
	VIRUS-VECTOR NEMATODES *Trichodorus* spp.	Use of loamless or sterilised composts. Otherwise sites soil-sampled to detect vector nematodes	Plants inspected for symptoms of virus infection	
HOPS	VIRUS-VECTOR NEMATODE *Xiphinema diversicaudatum*	Sites soil-sampled to detect vector nematodes	Plants inspected for symptoms of virus infection	

TABLE 1 (continued)

CROP	PEST	Time of inspection and measures taken: PRE-PLANTING	GROWING CROP	POST-HARVEST
STRAWBERRY	VIRUS-VECTOR NEMATODES *X. diversicaudatum,* *Longidorus elongatus,* *L. macrosoma,* *L. attenuatus*	Sites soil-sampled to detect vector nematodes	Plants inspected for symptoms of virus infection	
	STEM NEMATODE	Avoidance of sites previously cropped with alternative hosts	Plants inspected for symptoms of nematode attack	
	LEAF NEMATODES *Aphelenchoides fragariae,* *A. ritzemabosi*		Plants inspected for symptoms of nematode attack	
BLACKCURRANT	VIRUS-VECTOR NEMATODE *X. diversicaudatum*	Sites soil-sampled to detect vector nematodes	Plants inspected for symptoms of virus infection	
	LEAF NEMATODE *A. ritzemabosi*		Plants inspected for symptoms of nematode attack	
CHERRY AND PLUM	VIRUS-VECTOR NEMATODES PLUM - *X. diversicaudatum* CHERRY - *L. elongatus,* *L. macrosoma,* *X. diversicaudatum*	Sites soil-sampled to detect vector nematodes	Plants inspected for symptoms of virus infection	

The use of pesticides can mask the presence of nematodes by reducing their numbers and suppressing plant symptoms, without complete eradication. Therefore, pesticides are seldom used specifically to control nematode infestations in crops entered for certification although soil fumigants are used to control vector nematodes in some situations.

SPECIFIC MEASURES TAKEN TO REDUCE RISKS

Potato cyst nematodes

Sites for all grades of seed potatoes are soil sampled before planting to detect potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*; sites in which the nematodes are detected are rejected (Table 1). During growing crop inspection the roots of plants are examined for cysts if potato cyst nematode attack is suspected. However, population densities sufficient to produce symptoms in the crop would almost certainly have been detected by soil sampling unless the soil infestation were extremely localised. The restriction on the amount of 'dirt or other extraneous matter' (including soil) allowed with seed potatoes further reduces the risk of cysts of potato cyst nematodes being distributed with seed tubers.

Potato tuber nematode

To detect potato tuber nematode, *Ditylenchus destructor*, the only measure available is post-harvest bulb and tuber inspection (Table 1). The nematode is seldom detected in soil; it produces no foliar symptom in potato and no specific foliar symptom in iris.

Stem nematode

Races of stem nematode, *Ditylenchus dipsaci*, differ in their host ranges but are indistinguishable morphologically. Sites are not soil sampled but sites previously cropped with alternative hosts of the three races infesting strawberry are avoided (Table 1). Growing crop inspection of narcissus and strawberry should eliminate those stocks with visible symptoms of infestation and in narcissus this measure is supplemented by post-harvest dry-bulb inspection (Table 1).

Leaf nematodes

The leaf nematodes, *Aphelenchoides ritzemabosi* and *A. fragariae*, are rarely detected in soil samples and can be confused with fungivorous species which commonly occur in soil. Growing crop inspection of strawberry and blackcurrant to detect symptoms of attack is the sole measure specifically designed to eliminate infested stocks (Table 1).

Virus-vector nematodes

Species of *Trichodorus*, *Xiphinema* and *Longidorus* are readily detected in soil. To reduce the risk of virus transmission to the stocks, sites used for the propagation of the higher grades of narcissus, hops, soft fruit and top fruit are soil sampled before planting. Sites in which appropriate vectors are detected are normally rejected. This measure is further backed up by inspection of the growing crops for virus symptoms (Table 1).

LIMITATIONS OF SAMPLING

Where detection of the pest or the symptoms it produces in the crop is based on a sampling procedure it is not possible to guarantee that the site or the crop is free particularly where, as with the detection of nematodes in soil, the volume from which the samples are taken is very large. Table 2 shows the maximum chances of detecting nematodes in soil with a 500 ml soil sample as used in potato certification. The calculations on which Table 2 is based assume that there are two million litres of top soil per hectare (to 20 cm depth) and that the sampling method and laboratory examination procedures reduce errors to the minimum. In practice nematode aggregation reduces the probability of detection below Poisson expectation (on which Table 2 is based) and laboratory examination procedures are never 100 per cent efficient.

TABLE 2

Poisson probability of detecting nematodes in soil with a 500 ml sample (From Southey, in press)

Average field population		Percentage chances	
per 500 ml	millions/ha	of detection	of failure to detect
0.01	0.04	1	99
0.05	0.2	5	95
0.1	0.4	10	90
0.5	2	39	61
1	4	63	37
2	8	85	15
3	12	95	5
5	20	99	1

Table 2 shows that soil sampling of potential certification sites will mostly detect and therefore eliminate sites with large nematode populations but, will mostly fail to detect sites with small nematode populations.

However, despite this limitation, soil sampling increases the overall probability of planting on truly nematode-free sites (Cotten, 1979) and thus (a) reduces the chance of infection with nematode-transmitted virus and failure of the stock at growing crop inspection and (b) reduces the chance of potato cyst nematode being distributed with seed potatoes.

Nematode-transmitted virus infections of certified bulb, hop and fruit stocks which occur because nematode vector populations were undetected by soil sampling should be discovered, and the stocks eliminated during inspection of the growing crops, providing symptoms are visible. Visible infestation of stem nematode in narcissus and strawberry and of leaf nematodes in strawberry and blackcurrant should also be detected and the stocks eliminated at this stage.

For some nematode risks, post-harvest inspection provides further back-up for soil sampling or growing crop inspection so that, despite the limitations of individual safeguards, combinations of measures can reduce the risks from nematodes to insignificant proportions. For example, the restriction on the amount of dirt or other extraneous matter (including soil) permitted with marketed seed potatoes greatly reduces the risk of disseminating cysts of potato cyst nematodes with seed tubers. The equation $R = S_{max} \cdot N.P$ adapted from that of Southey (1979) can be used to calculate the number of cysts (R) per hectare that could be introduced into ware fields with lower grade (CC) seed potatoes which have been grown on sites in ware areas where the nematodes were present but undetected.

Where S_{max} = maximum tolerance for soil with seed potatoes (decimal proportion)
N = potato cyst nematode population at source (cysts/kg)
P = planting rate for seed potatoes (kg/ha)

The Seed Potato Regulations 1984 require that $S_{max} = 0.01$. For population densities of potato cyst nematodes which would be undetected 99 per cent of the time by soil sampling (Table 2), N = 0.02 (assuming a soil bulk density of 1 g/ml). Taking P = 2500, then R = 0.5 cysts/ha. Even with populations that would be undetected only 1 per cent of the time R is still relatively small at 250 cysts/ha. In areas where potato cyst nematodes have become established, infestation levels in ware fields may be counted in millions of cysts/ha before damage is apparent and the nematodes are readily detected by soil sampling (Table 2). In such cases, the addition of 0.5 or even 250 cysts/ha into a ware potato field with the seed at planting has no practical signficance for the health of that crop.

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PEST PROBLEMS ON IMPORTED PLANTING MATERIAL

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ABSTRACT

The increasing volume and changes in the pattern of plant imports are reviewed. Large numbers of species alien to Britain feed on plants in the exporting countries but many fewer have been found on imports and fewer still have become established in Britain. The influence of the narrow range of climates found in Britain is discussed together with that of the additional climates provided by glasshouses and other protected environments. Statutory measures to prevent introduction of alien pests include prohibition of imports of particular plants, pre-export conditions, pesticidal treatments and post-entry conditions. The wider responsibilities of importers and measures that they can take to supplement statutory measures are also discussed.

INTRODUCTION

Britain has a history of plant introduction spanning the centuries possibly from prehistoric times to the present day. Early introductions are difficult to date but it is clear that the rate of plant introduction has accelerated from the 17th Century onwards. This acceleration was at first in numbers of species with relatively small numbers of individuals being imported. However, in this century and particularly in the last few decades the pattern has expanded with the import of large numbers of individuals from relatively few species. Even so the number of species imported is still large. There are no precise statistics but from such records as are available in MAFF we estimate that more than 1500 different genera of growing plants are imported annually. Likewise precise numbers are virtually impossible to obtain but Table 1 has been compiled from the broad categories shown in the published import data and shows the change in pattern and growth in imports since 1974. For chrysanthemums, where the industry relies heavily on imports for high-grade propagating material, more than 80 million cuttings may be imported annually.

Many phytophagous insects and other invertebrates feed on these imported plants in their countries of origin. Just how many species is not known mainly because of taxonomic uncertainties and the labour and expense of preparing catalogues. However, the embryo database held in MAFF indicated, for example, at least 380 alien species feeding on apple and pear and 60 on chrysanthemum in temperate parts of the world, but these figures are likely to be substantial under-estimates.

PEST IMPORTATION AND ADAPTATION

The records of pests found on imported plants can be used to relate potential to actual arrivals. Over the last ten years some 225 pest species have been recorded. The sources of some of these are listed in Table 2.

TABLE 1.

Imports of plants into the United Kingdom (growing or intended for planting). Seeds and Bulbs are not included.

Plants	Source	Numbers of plants in thousands 1974	1979	1984
Azaleas	EEC	1 577	1 570	3 309
	EM	4	1	1
	Rest	15	<1	8
Roses	EEC	29 150	25 934	48 970
	EM	64	6	49
	Rest	<1	<1	1
Fruit trees	EEC	1 577	2 254	4 159
	EM	1 192	97	<1
	Rest	7	7	1
Other trees & shrubs	EEC	38 708	56 761	76 095
	EM	4 361	1 551	185
	Rest	149	84	614
Cuttings & slips*	EEC	24 559	44 355	127 419
	EM	2 491	5 346	698
	Rest	6 853	11 340	24 114
All other plants	EEC	2 228	7 302	18 255
	EM	28 895	73 977	33 080
	Rest	19 236	20 049	4 287

(EEC = European Community (The nine members as in 1974): EM = Other European and Mediterranean Countries; Rest = Rest of the world)

*Numbers estimated from weights

(source: Anon (1975-1985)

During the same period MAFF inspectors found more than 75 apparently undescribed species on imports which highlights the taxonomic problems.

A relatively small number of alien species apparantly transported to Britain by man have actually become established here although Carter (1983) suggests that only four of the 22 *Cinara* spp. recorded on conifers are native. Again it is not possible to produce a definitive list because many of the published new British records contain no information on the breeding status of the species and on their distribution abroad. Many

TABLE 2.

Sources of alien pests found on imported plants by M.A.F.F. Plant Health and Seeds Inspectors 1974-84

Source of plants	Number of species
Europe	38
Africa	67
North America	23
Central America	17
South America	14
Australasia	31
Far East	56
India	10
Middle East	7

(Source: Seymour *et al.*) (1975-185)

species with a continental European distribution were found for the first time in Britain during this century. Most may have been long established residents that had not been collected before but a few such as the Tortricids *Cacoecimorpha pronubana* and *Adoxophyes orana* were almost certainly new arrivals. Both these species spread rapidly within a few years of the first record. Both have been found on imports and it is impossible to know whether they became established from this source or by natural spread. It is only with the immigrants from more distant parts of the world that one can say with some certainty that they were transported by man. Short lists of established New Zealand, Far Eastern and American species are given in Table 3, but one must remember that an insect could conceivably have remained unrecorded in Britain and not have been described until transported elsewhere by man. Winter (1985) in discussing *Ips typographus* provides an example of the difficulty of determining whether an uncommon species is resident in Britain or not.

The paucity of recorded interceptions and of new establishments may be due at least in part to the standards of pest freedom to which imports have to conform to meet statutory and commercial requirements. Also, not all the species that feed on a particular plant can be transported in international trade, e.g. container-grown plants in full leaf and fruiting are likely to harbour more pest species than dormant bare-rooted plants or seed.

However, the most influential factor affecting the establishment is probably the "adaptation gap". Any alien species must be highly pre-adapted to living in Britain to have a good chance of establishing itself here. In particular pre-adaptation is necessary to the relatively narrow span of cool temperate climates found in the British Isles. This probably accounts for the few species that have become established compared with more than 1300 recorded for the continental USA (Sailer, 1978). Possible criteria for identifying the pre-adapted species have been discussed in more detail elsewhere (Baker and Bailey, 1979), but despite national and international attempts to list the most important species it is unlikely that they will all be identified.

TABLE 3.

Some alien phytophagous invertebrates established in Britain

Probably from New Zealand

HOMOPTERA
Coccoidea
Eulepidosaphes pyriformes
Leucaspis podocarpi
Noteococcus hoheriae
Trionymus diminutus

ORTHOPTERA
Phasmida
Acanthoxyla prasina
Clitarchus hookeri

COLEOPTERA
Curculionidae
Euophryum confine (1)

Elateridae
Panspoeus guttatus (2)

LEPIDOPTERA
Tortricidae
Epiphyas postvittana

Probably from North America (3)

DIPTERA
Cecidomyidae
Dasyneura gleditschiae

HOMOPTERA
Aphidoidea
Macrosiphum albifrons
Masonaphis goldamaryae
Illinoia lambersi
Wahlgreniella nervata
Adelges cooleyi
Pineus similis
Pineus strobi
Cedrobium lapportei

Cicadellidae
Graphocephala fennahi

HETEROPTERA
Tingidae
Stephanitis rhododendri

HYMENOPTERA
Torymidae
Megastigmus spermotrophus
Megastigmus atedius
Megastigmus pinus

Probably from Far East (3)

HOMOPTERA
Aphidoidea
Fimbriaphis wakibae
Macrosiphoniella sanborni
Takecallis arundinariae

Coccoidea
Pulvinaria regalis

LEPIDOPTERA
Gracillariidae
Caloptilia azaleella

Probably from Himalayan region

HOMOPTERA
Aleyrodidae
Dialeurodes chittendeni

(1) Feeds on dead wood (2) Feeding habits unknown
(3) Some species may have arrived via Europe

(Sources: Carter (1983), Owen *et al.* (1985), Williams (1985) and numerous papers in Entomologist's Gazette and other entomological journals)

Introduction with their host plant provides the best possible circumstance to enable immigrant species to found colonies. The host plant is a valuable item to be cared for by the grower. If pest infestation is not obvious pesticides might not be applied until the pest has dispersed.

Once introduced those pests that can survive outdoors have the opportunity to spread as widely as their host plant distribution and their own adaptability permit. An oligophagous species with good powers of dispersal, e.g. Lupin aphid *Macrosiphum albifrons* (Carter *et al.*, 1984), may be able to spread as fast as a polyphagous species with abundant wild or cultivated hosts but less effective dispersal abilities. However, the rate of spread of introduced species is little understood and the complex of factors that control spread may operate subtly. Some species appear to remain static in a relatively small bridge-head for many years before some unknown change permits further spread.

Glasshouses and other protected environments greatly widen the total range of climates in Britain but these environments are usually small in size and often spatially isolated from one another although the isolation may not be great in highly concentrated areas of protected cropping. So, unless there is ready recruitment from outside, the restricted pest populations that they sustain are particularly vulnerable to pesticides, temperature regimes and cropping. This may be why a number of alien species that have been found under glass (Table 4) appear to have succumbed readily to eradication measures. Among the most favourable sites for colonisation are glasshouses containing mother stock for propagation, botanic gardens and showhouses containing long-lived specimen plants.

TABLE 4.

Alien pests found as temporary colonies under glass and eradicated

ACARI	LEPIDOPTERA
Diptacus swensoni	*Glyphodes stolalis* (complex)
Panonychus citri	*Opogona sacchari*
	Cornutiplusia circumflexa
DIPTERA	*Helicoverpa armigera*
	Spodoptera eridania
Liriomyza trifolii	*Spodoptera littoralis*
Aschistonyx eppoi	*Spodoptera litura*
	Dattinia sp.
HOMOPTERA	
	NEMATODA
Dialeurodes citri	
Dialeurodes citrifolii	*Radopholus similis*
Aphis citricola	
Aphis nerii	
Rhopalosiphum rufiabdominale	

(Sources: Powell (1979) and later M.A.F.F. records)

Although glasshouses might seem to provide favourable environments there are indications that some species that are outdoor pests in tropical and sub-tropical areas may not readily become glasshouse pests. For example, in countries where it occurs, the whitefly *Bemisia tabaci* has not become as important a glasshouse pest as *Trialeurodes vaporariorum*. The reasons for this are not obvious.

CONTROLLING PEST IMPORTATION

Statutory control of imports is one measure that can be used to supplement the British climate in preventing the establishment of alien pests. The subject was treated in some depth by Southey (1979) so it will only be reviewed briefly. Statutory measures can be grouped under four main headings namely prohibitions, treatments, pre-export conditions and post-entry conditions.

Prohibiting the import of particular plants may appear to some to be a draconian measure though against major pests specific to important crops prohibition can be an effective measure. It may also be appropriate to prohibit larger groups of plants (e.g. Gramineae) that contain crop plants from certain parts of the world: for example from areas with a large indigenous fauna alien to Britain or from areas where the fauna is little known but likely to contain potential pests. Prohibition may be particularly useful in this context when linked to powers to allow imports under licence or permit. Prohibited plants can then be imported with safeguards that may be too experimental or too complicated to be embodied in legislation. If experience shows the safeguards to be effective or even unnecessary the legislation can be amended accordingly.

The key sections of most plant health import regulations are the pre-export conditions. The objective is that only healthy pest-free plants shall be exported. Almost all other measures are employed in default of this. Although very precise measures may be required to be applied before export, their application is almost entirely in the hands of the exporting country. Documents confirming that the measures have been taken have to be accepted on trust but this can be enhanced by knowledge of the efficiency and competence of the issuing Plant Health Authority. The effectiveness of the pre-export measures can be monitored to a certain extent by inspection of samples on arrival. However, it must be emphasized that visual inspection by itself can very rarely be an effective safeguard for obvious statistical and logistic reasons.

Pesticidal treatments may be an effective way of freeing imported plants from pests whilst causing little interference with trade. The ideal treatment must meet three criteria. There must be a sufficient safety margin between killing the pests and phytotoxicity. Distribution of the treatment through the consignment must be very even. The treatment must not be used as a routine control measure in the production area prior to export. Both cold storage and fumigation can fit two out of the three criteria but they tend to be phytotoxic. With other treatments, even distribution may be more difficult to achieve and the possibility is greater that the pests may be resistant to the pesticides used. However, it must be remembered that regulatory treatments of imports are unlikely to enhance resistance *per se* as there is little return of the treated pests to the source populations abroad.

It is possible to apply treatments or to retain plants in quarantine after arrival as is done in a number of countries with wide climatic spans and a correspondingly wide range of crops vulnerable to alien pests, particularly Australia and USA. Because post-entry conditions such as these diminish the onus placed on the exporter to provide healthy plants they are not normally used in the UK. The only exceptions are small imports of high-value high-risk plants imported under special licence.

Although the import regulations may play a large part in ensuring that imported plants are free from pests, other pressures and other responsibilities are involved. Under British Law it is the individual citizen who is responsible for conforming to the regulations. This responsibility needs to be seen in the wider context of not only protecting the agricultural, horticultural and forestry industries but of protecting all consumers of plants including gardeners and users of plantings in public amenity and recreational areas. Outbreaks as dramatic as Dutch Elm disease are fortunately rare and there are few alien pests likely to produce comparable damage. But there are alien species that would be significant additions to the pest burden. Many others of minor significance individually would assume greater importance if several were to be introduced.

The importing grower can supplement official activity to ensure that his plants remain pest free. Firstly he can make a critical examination of plants on receipt and take a tough line with the exporter if pests are found. Secondly, following the examination, a routine pesticide treatment should be considered as a relatively inexpensive insurance against future problems. Thirdly, if a pest is found that is unfamiliar, unusually damaging, or does not respond to treatment as expected, it is important to get the pest expertly and promptly diagnosed however limited the incidence may be.

The methods of propagation used by both the exporting and importing growers have a significant effect on the risk of plants acquiring colonies of pests. Moves to raise the productivity of an enterprise by reducing the period of propagation and also to raise the health standard of the mother stock can reduce the risk of infestation at times to a negligible level. Relevant techniques include propagating temperate plants under cover, using precisely defined and sterile growing media and micropropagation. Although not a complete panacea, micropropagation has great potential for producing large numbers of pest-free plants. This is because the size of the propagules is small in relation to the size of most invertebrate pests so the pests or the damaged plantlets are easily seen and eliminated. A few pests such as immature aphids, tetranychid, tarsonemid and eriophyid mites and nematodes could escape notice. With good hygiene, isolation and screening precautions, plants in the bottle stage should be unlikely to acquire further infestation though some pests, e.g. dispersing thrips, show considerable powers of penetrating artificial barriers. The risk of infestation increases again when the plantlets are set out in conventional growing media so the bottle stage is particularly safe for use in international trade and official regulations should not inhibit its use. Indeed some countries now include in their legislation special conditions for micropropagation.

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EXPORT INSPECTION OF PLANT MATERIAL FOR PESTS IN THE NETHERLANDS

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ABSTRACT

The phytosanitary export inspection procedures of plant material in the Netherlands are described. Information is given on field inspection, pre-export inspection and on the Services involved in these inspections. The significance of quarantine and quality pests is discussed. Examples of special export inspection measures for quarantine pests important to the UK are provided (e.g. Liriomyza trifolii).

PHYTOSANITARY INSPECTIONS IN GENERAL

Phytosanitary import and export inspection are based on the FAO International Plant Protection Convention (1951). In this Convention the model of the phytosanitary certificate is laid down. On this certificate the Plant Protection Service declares that the plants have been inspected and found free from quarantine pests, practically free from other injurious pests and are believed to conform with the current phytosanitary requirements of the importing country.

Consequently a distinction should be made between "quarantine" pests and "other" pests or so-called "quality" pests. Quarantine pests are not present in the importing country, or are not widely distributed there and are subject to domestic quarantine requirements. They are of potential economic importance. Countries should publish a list of quarantine pests in their import requirements. Consignments must be free of quarantine pests (zero-tolerance).

Other pests or quality pests are already present in the importing country and are widespread or "cosmopolitan" in distribution. They mainly affect the quality of the consignment itself, are generally capable of causing losses in yield, but they are not a serious risk for the country's agriculture. Often they are covered by a national certification scheme. At export inspection, consignments must be substantially free of quality pests (small tolerance).

The phytosanitary import requirements of EEC-member countries are harmonized by the EEC Plant Health Directive *. The aim of this Directive is to make common protection arrangements against the introduction of quarantine pests from non-member countries and also to re-organize and facilitate plant health inspections on intra-Community trade. In this Directive 32 insects and 7 nematodes are listed as quarantine pests for all member countries together with 5 additional insects specifically for the UK and a few other countries.

* Directive 77/93/EEC. Official Journal of the European Communities No L26, 31.3.1977, p. 20-54.

PHYTOSANITARY INSPECTIONS IN THE NETHERLANDS

The inspection of plant material in the Netherlands is based on a growing season inspection or "field inspection" and on a pre-export inspection.

The field inspection of the most important planting material is done by the 5 General Dutch Inspection Services (Table 1). The certification schemes for these planting materials are obligatory. It is not allowed to sell planting material of these crops which does not meet our national requirements.

TABLE I

The 5 general Dutch Inspection Services and their area of activity.

Service	Planting material
NAK	seed potatoes and agricultural seeds
NAKB	fruit trees, strawberry plants, some conifers and some broad leaved and ornamental trees and shrubs.
NAKS	carnation, chrysanthemum, cyclamen, freesia, nerine and pelargonium
NAKG	vegetable and flower seeds, seed shallots, onion sets
BKD	flower bulbs.

The aim of these Inspection Services is to ensure that only planting material that meets high standards of quality and health is marketed. They carry out field inspections, take samples for testing in the laboratory and accept only lots complying with specified standards and requirements.

The inspection on health concerns:
. practically free of quality pests up to the classification standard.
. free of quarantine pests.

The Inspection Services are semi-official, working under the supervision of the Minister of Agriculture and Fisheries. Experts of the Plant Protection Service are members of the Board of each of these Inspection Services. This line of communication ensures that the standards of the Inspection Services meet both the national plant health requirements as well as international standards, in so far as the importing country does not have special quarantine requirements.

If field inspections are required for crops other than these mentioned above, then these are undertaken by the Plant Protection Service itself.

Pre-export inspections of planting material are done by the Plant Protection Service at the premises of the exporter before the plants are packed. The Headquarters of the Service, which is a department of the Ministry of Agriculture and Fisheries, is at Wageningen (200 staff). There are 14 district offices spread over the country with 200 inspectors in total. The inspectors are supplied with a loose-leaf instruction book which sets down the instructions on general health standards (practically free of quality pests) as well as the specific requirement of the importing country (free of quarantine pests). The 16 technical departments at Headquarters

are either related to crops (horticulture, arboriculture, agriculture), to diagnosis (entomology, mycology, bacteriology, nematology), or to special activities (import and export-inspection, quarantine).
Samples taken by the inspectors during their inspections are sent to the specialists of our own Service at Wageningen. The inspector will receive a reply as soon as possible. The organization scheme of the Service is set up in such a way that lines of communication and decision are short. This reflects the importance of imports and exports for our country.

A high percentage of the production of plant material in the Netherlands is exported (50 - 70%). Therefore planting material is inspected in such a way that all lots that pass inspection are in principle acceptable for the national market as well as for export. In cases where the importing country requires special health measures, additional inspections have to be made.

EXAMPLES OF SPECIAL INSPECTION FOR PESTS IMPORTANT TO THE UK

Leptinotarsa decemlineata

After the second world war, when during hot summer periods the aerial immigration of Colorado beetles from east and south across our frontiers increased, we found that the eradication campaign was to no avail. Nowadays, we know by experience that the climatic conditions in our country are only favourable for the development of the beetle during short periods. In the western and northern parts of the country, where the main potato production area is located, such periods are particularly rare. Besides most potato crops are regularly sprayed for other reasons (aphids, Phytophthora), so that, if necessary, the beetle can be controlled by additional pesticides applied with the routine treatments.

The beetle never causes crop losses of any importance in our country. However, many plants and products have to be exported to countries where Colorado beetle is regarded as a quarantine pest. For this reason the control of the beetle is regulated by a domestic quarantine law. Farmers are obliged to control the beetle. The Plant Protection Service checks fields during critical periods.

Beetles on volunteer potato plants in grain fields are difficult to control. In the past there have been a few problems when grain for sowing or consumption was exported to the UK shortly after harvesting, without careful cleaning. The instructions have now been improved, and no further cases have been reported.

Liriomyza trifolii

Several years ago the pest was introduced into our country. Eradication programmes were set up but failed. Research on the improvement of control measures was stimulated. The Dutch growers of chrysanthemum plants, by following the measures recommended by the Extension Service, have now succeeded in keeping their produce free or practically free from leaf-miner.

In 1980 the UK plant health authorities informed our Service that during import inspection of chrysanthemum cut flowers some lots infested with L. trifolii had been found. More stringent pre-export inspections were

considered necessary to avoid severe import-restrictions. After bilateral discussions, a new pre-export procedure for this leaf-miner and for chrysanthemum white rust was agreed. Later this procedure was improved, based on further experience.
The new system is called the "green corner". For all consignments of chrysanthemum pot plants and cut flowers destined for countries where L. trifolii is a quarantine pest, the green corner procedure is followed. Chrysanthemum cuttings must have been certified by the NAKS, which means that the cuttings and their mother plants originate from a nursery found free of the pest.

The green corner procedure is based on 3 inspection steps:
. registration and inspection of the nursery;
. inspection at the auction; and
. pre-export inspection.

Growers wishing to supply chrysanthemums for the green corner must apply to their local auction to be registered. In the month prior to registration, the nursery is inspected by inspectors of both, the auction and the Plant Protection Service, and must be found completely free from evidence of leaf-miner infestation. The application inspections are followed by regular fortnightly inspections. The approved nurseries are recorded on a "green list". Any changes are communicated immediately by telephone. The list is updated weekly.
If at any stage infected plants are detected, the nursery is removed from the list for at least 4 weeks. After that period the nursery can re-apply for registration.

After arrival in the delivery hall of the auction, pot plants and cut flowers supplied by the approved nurseries are inspected by auction inspectors. Boxes containing produce found free from leaf-miner (and white rust) are marked with a green sticker upon the suppliers label. The date of supply is printed on the green sticker.

At pre-export inspection for the UK, undertaken by the Plant Production Service, only chrysanthemums produced by green corner nurseries are accepted. The exporter is required to draw up a packing list on which all lots are specified by number of bunches or plants, cultivar, name and number of the supplying nursery. Each lot is inspected intensively.

The costs of controlling pests and diseases in the year-round culture of chrysanthemums are about 4% of the total production costs. Besides leaf-miner (and white rust) some other pests and diseases must also be controlled. It is possible to mix pesticides for controlling all these parasites. This means that the extra costs of controlling leaf-miners (and white rust) are mainly the initial expense on pesticides to control these parasites or only 2% of the total production costs.

There is practically no difference in costs of control for growers participating in the green corner in comparison with those who are not interested in the programme. Many growers remaining outside the green corner deliver pest-free produce to the auction, but do not like the continual inspection pressure. Depending on the season, 50 to 70% of the nurseries are registered in the green corner.

Another host plant for L. trifolii is Gypsophila. Last year we started a comparable inspection procedure for cut flowers of Gypsophila to guarantee the export of leaf-miner free produce.

Opogona sacchari

The banana moth can be introduced with many ornamental plants of tropical origin (Dracaena, Yucca, Strelitzia, etc.).
The pest was intercepted for the first time in 1971, followed by several further infested consignments.
As an infestation with O. sacchari is difficult to detect at import inspection, imported host plants are held under post-entry quarantine in the grower's glasshouses for 3 months.
Infested lots are returned to the exporting country, destroyed or put under special quarantine measures until the infestation has been eradicated. The UK and the Netherlands have proposed this pest for the EEC list of quarantine pests.

Globodera rostochiensis and G. pallida

In the Netherlands, all fields on which the farmer wants to grow planting material (seed potatoes, nursery stock, flower bulbs etc.) should be found free from potato cyst nematode by the Plant Protection Service. This system avoids sampling and testing for this nematode at pre-export inspection. At import inspection of planting material arriving in the Netherlands, soil samples are taken and checked in the laboratory. Infested lots have to be cleaned or returned.

Ditylenchus dipsaci

In the Netherlands the tulip stem nematode is considered to be a quarantine pest. Reasons are the importance of flower bulb production (14,000 hectares) and the difficulty to control this pest, especially on tulips and hyacinths. The result of the eradication campaign is that it is very seldom that an infested lot is found. Such lots have to be destroyed. It means also that all imported lots of flower bulbs are checked for this nematode and have to be free of the pest.

Phthorimaea operculella

The potato tuber moth does not occur in the Netherlands. Climatic conditions in our country are not favourable for this pest. However, during the summer the moth can spread from imported, infested lots of early potatoes to potato fields surrounding the packing station. So the UK as well as the Netherlands consider tuber moth as a quarantine pest. Imported early potatoes are inspected carefully for tuber moth.

CONCLUSION

In the Netherlands the general health standards for planting material are set at a high level. Plants can thus be exported without additional field and pre-export inspection. However, sometimes the importing country is in a special phytosanitary situation and for that reason requires reasonable extra plant health safeguards. In these circumstances, taking into account the significance of the export of these plants for our country and after consultation with grower and trade organizations, the Plant Protection Service undertakes additional export inspection measures. These are illustrated by a few examples with respect to the special import requirements of the UK.

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THE ROLE OF PLANT RESISTANCE TO PESTS IN THE PROVISION OF HEALTHY PLANTING MATERIAL

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ABSTRACT

The use of pest-resistant cultivars of temperate crops makes a significant contribution to the production of healthy planting material and to ensuring continued plant health. Examples are given of the benefits of utilising resistant cultivars on their own or in combination with other approaches to pest control. To increase awareness of their potential, the different methods of deploying resistant cultivars are described. As with all approaches to pest control the development and use of resistant cultivars has its drawbacks. However, these problems are far outweighed by the benefits of reducing costs, of reducing pest populations and protecting the environment. Prospects for increasing the use of resistant cultivars in the future are great providing that the latest techniques are adopted and that there is full collaboration between scientists, seed companies, advisers and growers.

INTRODUCTION

Amongst the many different approaches to the problem of producing pest-free planting material, host plant resistance is one of the most attractive control methods yet it has received comparatively little study. By far the greatest effort in tackling pest problems has been invested in the research, development and use of pesticides. It is true to say that the production of pest-free plants is, in the majority of cases, dependent on the use of chemicals and this situation is likely to continue for many years to come. However, as alternative approaches are investigated and developed the growers' dependence on pesticides should be reduced. Indeed, pressures from many different quarters are being brought to bear to encourage the research and adoption of alternative approaches. Only in recent years has a concerted effort been made to monitor and predict pest populations, make use of cultural control measures, utilise natural enemies and explore the resistance of crops to pest attack. The purpose of this paper is to increase awareness of the potential of plant resistance to indicate what are the distinct benefits of this approach. In addition, some idea is given of what is currently possible, what are the problems and what are the future prospects of this approach to the production of healthy planting material. Strategies for deploying resistant cultivars are also discussed.

The concept of 'pest-free' planting material is one which should be questioned. The market demands for pest- and blemish-free produce are quite unrealistic, practically impossible to achieve, and are unlikely to be achieved in the future especially as pressures increase from all sides for a reduction in pesticide use. Even the most satisfactory methods of applying

insecticides to control vegetable pests lead to non-uniformity which leaves a significant fraction of the crop unprotected (Suett & Thompson, 1985). Similarly, very few resistant cultivars result in a pest-free situation. However, resistance does not have to exist at levels of immunity to be of value; indeed immunity is exceedingly rare. Partial resistance can be of great value particularly when used in combination with other control measures (see below).

The concept of healthy planting material should also be debated. Crops do not necessarily have to be pest-free to be healthy. In certain cases small pest infestations can actually lead to more satisfactory marketable yields of a crop. For example, light, cyst nematode attack on potatoes (Jones, 1976) and tomatoes (Hesling & Ellis, 1972). With these crops light infestations promote root proliferation which can increase water and nutrient uptake. Provided that resistant cultivars reduce pest numbers to reasonable levels then slight damage can, in many cases, be tolerated. The term 'healthy planting material' can have more than one meaning; at this symposium we are mainly concerned with the pest- and disease-free aspects but it is worth stressing that planting material must produce healthy food for human consumption. Imminent legislation and the increasing demand for organically-grown produce are aiming to make both crops and the environment healthier for humans. These pressures will discourage chemical use and encourage non-chemical methods such as the use of resistant crop cultivars. However, all approaches to pest control have their drawbacks and in a few cases resistant breeding lines have been developed which possess compounds which are definitely not healthy for human consumption.

BENEFITS OF PLANT RESISTANCE

The use of resistant cultivars has many advantages over other approaches to pest control. As a result of the wise deployment of resistant cultivars many important pests of temperate crops have been reduced to levels where they are no longer of economic importance. The great advantages to the grower are the lower costs of producing healthy planting material and the ensurance of continued plant health. The seed of resistant cultivars costs very little extra compared with that of susceptible cultivars. The reduced use of chemicals and spraying equipment saves both time and money. The grower has less contact with toxic chemicals and this can be of particular benefit where planting material is raised in a protected environment in which people work and handle plants. Glasshouse and polythene-clad structures may have to be evacuated following the application of certain chemicals; these situations could be avoided. Pesticide residues in the crop and soil are also reduced which not only makes the planting material more healthy but also reduces environmental pollution and the associated harmful effects on natural enemies of pests and other wildlife.

Two significant benefits appreciated by the crop protection expert are firstly the compatibility of plant resistance with other approaches to pest control; in this way resistant cultivars make a significant contribution to an increasing number of pest control programmes (see below). Secondly, resistant cultivars can provide a long-term answer to some pest problems. The reduction in pest populations is constant, cumulative and density independent. There are many examples of the durability of resistance. The

grape phylloxera, Phylloxera vitifoliae, has been kept in check for well over 100 years by the use of resistant vine rootstocks. The resistance in several lettuce cultivars to lettuce root aphid, Pemphigus bursarius, has provided a satisfactory method of control for more than 15 years. Similarly, the resistance to the woolly apple aphid, Eriosoma lanigerum, which originated in the apple cultivar 'Northern Spy', has been effective for more than 100 years. This ensurance of continued plant health is lacking when most pesticides are used and lacking in some cultural and biological control programmes. The enormous benefits from growing resistant cultivars have been calculated in economic terms (Luginbill, 1969) and their contribution to pest control programmes reviewed by Russell (1978) and Maxwell & Jennings (1980).

COMPATIBILITY OF PLANT RESISTANCE WITH OTHER APPROACHES

Ideally a resistant cultivar should be immune to a range of pests and diseases and therefore provide a complete answer to the growers' problems. However, immunity is very rare, the root aphid resistance in lettuce being an excellent example, and it is more likely that partial or moderate levels of resistance will be available. Fortunately, even partial resistance can contribute significantly to the production of healthy planting material. Unlike most applications of pesticides and certain other techniques the growing of resistant cultivars is compatible with other pest control measures. This distinct advantage is unlikely to be reduced by the technological revolution which is taking place in the production of planting material. Cultural control techniques are usually complemented by the growing of resistant plants. For example, crop rotation and the use of resistant cultivars are extremely effective in controlling cyst nematodes on potatoes and barley in Europe. Attempts to avoid peak pest infestation by altering cropping schedules is complemented by growing resistant cultivars. For example, a combination of a partially-resistant carrot cultivar and careful choice of sowing and harvest dates can greatly reduce the severity of carrot fly damage (Ellis et al., 1986).

The growing of resistant cultivars is compatible with the biological control of pests. The absence of pesticides and other side effects favours the natural enemies. Resistance in the plant has adverse effects on the behaviour and development of pests exposing them to biological control agents for longer periods. In some cases resistance is also less affected by environmental changes than the predators or parasites. This is one of the reasons for developing integrated programmes involving plant resistance and natural enemies for the control of two-spotted spider mite, Tetranychus urticae, on cucumber and the glasshouse whitefly, Trialeurodes vaporariorum, on tomatoes (de Ponti, 1980). Biological control of cabbage aphid, Brevicoryne brassicae, on Brussels sprouts was enhanced by the use of resistant cultivars which were found to be more attractive to natural enemies than susceptible cultivars (Way & Murdie, 1965).

Insecticide use is compatible with the growing of resistant cultivars and, in some cases, an additive effect has been demonstrated. For example, with moderate infestations of certain caterpillar pests Chalfant & Brett (1967) found that partially-resistant cabbages responded more favourably to insecticide treatments than susceptible cultivars. Partial resistance in itself can lead to a marked reduction in the dose of insecticide required to

provide satisfactory control of a pest. In the control of carrot fly, Psila rosae, one third of the recommended dose of insecticide was required to control this pest on a partially-resistant cultivar compared with that required on a susceptible cultivar (Thompson et al., 1980). Reduced doses of chemical could well delay the development of pesticide-resistant races of a pest.

STRATEGIES FOR THE DEPLOYMENT OF PLANT RESISTANCE

The effectiveness of any approach to producing healthy planting material depends on its wise deployment. This is certainly true of resistant cultivars (Kennedy, 1984). To utilise plant resistance effectively the grower must be aware of the principle pests involved and be aware of the resistant cultivars available. In the UK, ADAS list the resistant cultivars available in certain of their publications (Anon., 1982). There are numerous ways in which plant resistance can be used to good effect. As mentioned above, ideally plant resistance should provide the principal control method. High levels of resistance to certain aphids have been bred into raspberry cultivars (Keep, 1981) and lettuce cultivars (Dunn & Kempton, 1974); insecticides are not required for these pests. High levels of resistance to another aphid pest of lettuce have been discovered and are being exploited (Eenink & Dieleman, 1984). In other cases a high level of resistance exists to one of several closely-related pests or to one but not all biological races of a pest. This situation does not preclude the use of a particular resistant cultivar. For example, the potato cultivar 'Maris Piper' bred for its resistance to the cyst nematode Globodera rostochiensis, is not resistant to the closely-related species G. pallida or indeed to all the races of G. rostochiensis. Nevertheless, through wise deployment 'Maris Piper' makes a valuable contribution to control of potato cyst nematodes and in 1983 accounted for 24% of the area of maincrop potatoes planted by registered producers (Anon., 1984). A rational scheme for utilising resistant potato cultivars has been devised (Jones & Jones, 1984). To overcome the problem of biological races it is necessary to introduce different genes for resistance into planting material and this has been done in several cases, for example in breeding for resistance to raspberry pests (Keep, 1981). A high degree of success has been achieved in breeding sweet potatoes for planting in temperate regions which possess resistance to a range of insects, nematodes and diseases (Jones et al., 1981) culminating in the development of new cultivars (Jones et al., 1985).

Resistance to root pests and diseases can be used to good effect in the form of grafted planting material. The resistance in vine rootstocks to the grape phylloxera is an excellent example. Also, in tomato growing, resistant rootstocks provide an answer to several important soil-borne pest and disease problems. Resistant cultivars can make a significant contribution to the problem of virus diseases in certain crops. If a high levels of resistance occurs to the vector then a virus problem can be reduced. This is the objective of studies of resistance in blackcurrant to the gall mite, Cecidophyopsis ribis, which is the only known vector of reversion virus in the crop (Knight, 1981). Certain melon lines are resistant to the melon aphid, Aphis gossypii, and this resistance prevents virus transmission by the pest (Lecoq et al., 1984).

DRAWBACKS OF THE PLANT RESISTANCE APPROACH

Breeding resistant cultivars of plants is a slow and expensive process, the development of new agronomically-acceptable cultivars taking in most cases at least 10-15 years. The process does not end with the release of a single resistant cultivar either, as market demands for what is acceptable in a crop change. In addition there is always the possibility that races of the pest will arise which can overcome the resistance in the cultivars that are available. Fortunately, in most pest/resistant cultivar interactions the development of resistance-breaking races has not taken place or if it has it has taken a long time, time enough to allow the exploitation of alternative genes for resistance. The problem of variable pests has been reviewed recently (MacKenzie, 1980). The process of breeding new cultivars can be shortened by adopting new techniques; for example in breeding carrots resistant to carrot fly, Psila rosae, the time taken to develop inbreds has been halved by manipulation of the techniques for raising plants and the environment in which they grow (Ellis et al., 1983). To achieve a high level of resistance in a new cultivar may mean sacrificing full yield potential, a course of action which is not popular with the producer. Most people's concept of resistance is immunity and it may be difficult to convince a grower of the potential of partial resistance and to accept low levels of pests and their damage. Similar problems have been faced when introducing biological control agents for the suppression of pests; in the latter circumstances the pest itself as well as the natural enemies may have to be released into the crop. Care is required in developing resistant cultivars to avoid introducing poisonous substances from wild crop relatives into new breeding lines. Analysis of breeding lines is therefore necessary to ensure their suitability for human consumption. Quite unexpected drawbacks can arise with resistant cultivars which can detract from their value. It has been discovered that certain lettuce cultivars resistant to lettuce root aphid, Pemphigus bursarius, are rejected by some consumers because the cut stems of the plants turn brown after harvest; this browning is associated with the high levels of phenolic compounds which confer resistance to the pest. Problems may arise in the selection procedures which can hamper progress in breeding for resistance; in these cases either further research is required to solve the problem or alternative procedures have to be adopted (Ellis et al., 1985). In most situations resistant cultivars alone cannot solve all the pest and disease problems of producing healthy planting material. The use of resistant cultivars has to be integrated with chemical and other control measures.

FUTURE PROSPECTS

Experience has shown that in most pest/temperate crop situations resistance exists and can be exploited. As a result there are numerous examples of the success in utilising resistant cultivars. It is not just economic benefits which should be counted but also the other important advantages described above. These advantages will become even more important in the future. In the past, plant breeders have strived to increase crop yields, often at the expense of resistance to pests and diseases. In the future a different policy must be adopted and one which is nearer to that prevailing in nature, that is, to increase pest and disease resistance at the expense of some loss in yield. This policy could be aided if seed companies created the conditions in which resistance could manifest itself instead of providing complete chemical protection against pests and diseases for seeding crops. Screening of breeders lines should be attempted, not

only to identify pest- and disease-resistant material but also to avoid the selection of ultra-susceptible lines for use in cultivar production. To speed up the identification and development of plant resistance the latest techniques will need to be utilised including chemical assays for assessing resistance of plant material, genetic engineering and tissue culture for exploiting genes for resistance and the use of multilines in the deployment of resistance. Progress can only be made by collaboration between scientists, seed companies, advisers and growers. The full potential of plant resistance in the provision of healthy planting material will then be realised.

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5.
Pesticides

Chairman	:	Professor J. M. Hirst
Session Organisers	:	R. M. Perrin
	:	A. R. Jutsum

THE USE OF PESTICIDES DURING PLANTING AND ESTABLISHMENT OF OILSEED RAPE, POTATO AND SUGAR BEET IN THE UK.

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ABSTRACT

Healthy, economically viable crops are grown only from healthy planting material. The cost of producing the material can be wasted if pests are allowed to remove or damage seeds and seedlings when this could be prevented or reduced. The usage and value of applying insecticides, molluscicides, and nematicides during early growth stages of oilseed rape, potato and sugar beet are examined and possible developments discussed.

INTRODUCTION

The expense and effort of producing healthy seed and planting material can be wasted if precautions are not taken to ensure that as many viable plants as possible are obtained from the material. This paper reviews the use of pesticides in order to establish crops with sufficient plants to give acceptable yields. Only pesticides (insecticides, molluscicides and nematicides) which act against invertebrate pests are considered. Pesticides which are applied during the early stages of crop development but intended for pests which attack later are also included. The important pests of oilseed rape, potato and sugar beet, the recommended chemical control methods and pesticide usage are examined. Some of these pests may be controlled by non-chemical methods. Cultural and biological control techniques can be integrated with chemical control to produce a practical, economical pest control system.

CROP HUSBANDRY AND PRODUCTION

Oilseed rape, potato and sugar beet are important in many arable farming enterprises as break crops and/or cash crops but they differ in several respects which have implications for the control of pests up to plant establishment. Oilseed rape and sugar beet have small seeds which produce single shoots. The true seed of the potato is similar but the 'seed' tuber planted for commercial crops is relatively large and capable of producing several shoots. Wireworms (*Agriotes* spp) and slugs (usually *Arion* and *Milax* spp) can tunnel into seed tubers, allowing access for diseases and secondary pests such as millipedes, but the large food reserve that a tuber has, and its ability to produce further shoots if some are removed, allows it to compensate for pest damage. Pests that attack UK potato crops before plant emergence are usually insignificant and insecticides and nematicides applied at planting are aimed at controlling those pests that attack later growth stages.

Most oilseed rape in the UK is autumn-sown, in contrast to the spring-planting/sowing of potato and sugar beet, and fits well into predominantly cereal rotations. The optimum plant population at establishment is 100-110 plants per m^2, to give about 80 per m^2 by spring, although as few as 9-10 established plants per m^2 can give an acceptable yield (Ward *et al* 1985). Narrowly-spaced rows (25cm) are common.

In contrast, sugar beet is usually precision-drilled to a stand on wide seed spacing and rows (Dunning and Byford 1983) to give an optimum plant population and leaf area after establishment are critical components of yield (Jones et al 1955, Scott and Jaggard 1978). Recent cultural changes, such as wide seed spacing and increased use of herbicides, which reduce the number of alternative host plants for pests, tend to intensify seedling pest problems (Hull 1978). The relative importance of seedling pests is reflected in the use of an insecticidal seed treatment on all sugar beet seed and the number of insecticides available for application at drilling.

PESTICIDE USAGE

There seems to have been an upward trend in the usage of pesticides in general recently (Kornberg 1979) but on arable crops (excluding cereals and grass) the trend has been inconsistent. Between 1977/79 and 1982 the area treated with insecticides and nematicides decreased from 88 to 73% of the area cropped but that treated with molluscicides increased from 3 to 18% over the same period (calculated from Sly 1984). However, the amount of insecticides and nematicides (kg. a.i/ha) increased from 0.7 to 0.9 but for molluscicides decreased from 0.6 to 0.2.

Since the most recent ADAS Pesticide Survey on arable crops (1982), more synthetic pyrethroids have entered the UK market and recommended uses for them have increased, especially on oilseed rape.

Table 1

Use of insecticides, nematicides and molluscicides in England and Wales, 1982 (based on Sly 1984).

	Oilseed rape		Maincrop potato		Sugar beet	
	Area treated (000's ha)	% crop treated	Area treated (000's ha)	% crop treated	Area treated (000's ha)	% crop treated
Organochlorines	43.7	21	1.9	6	33.4	22
Organophosphates	69.2	40	43.4	32	20.9	10
Carbamates	93.6	51	70.3	55	121.9	61
Pyrethroids	0	0	*	1	0	0
Seed treatments	131.2	76	0	0	204.1	99

* small but unquantified.

Oilseed rape

In 1982, an oilseed rape crop received an average of 1.95 insecticidal/molluscicidal treatments (calculated from Sly 1984). Gamma-HCH, as a seed treatment, and methiocarb were the most frequently used chemicals. Gamma-HCH was also applied as a spray, from emergence until December, to control cabbage stem flea beetle (Psylliodes chrysocephala), a major pest of autumn-sown oilseed rape in southern

England. This insecticide is also the recommended treatment, applied as a seed treatment or spray, for other flea beetles (Phyllotreta spp). However, these flea beetles are pests of spring-sown rape only (Gladders and Gould 1983, Ward et al 1985). In view of the problems encountered with cabbage stem flea beetle damage on winter crops that received a gamma-HCH seed treatment, it is unlikely that the treatment is contributing significantly to the control of this autumn pest. The continued use of gamma-HCH seed dressing on autumn-sown oilseed rape is probably unnecessary and possibly detrimental to the environment.

Table 2

Winter oilseed rape - major pests and recommended treatments at drilling or establishment.

Pest	Chemicals
Cabbage root fly	carbofuran, fonofos, phorate
Cabbage stem flea beetle	carbofuran, cypermethrin, deltamethrin, fenvalerate, fonofos, gamma-HCH, permethrin, phorate, pirimiphos-methyl, WL 85871 ('Fastac')
Rape winter stem weevil	carbofuran, cypermethrin, deltamethrin, fenvalerate, gamma-HCH, phorate, WL 85871
Slugs	metaldehyde, methiocarb copper sulphate mixtures

Methiocarb, the only carbamate recorded by Sly as being applied (Table 1), is recommended to reduce damage caused by slugs (mainly Deroceras and Arion spp) to the seed and seedling (Table 2). At least one product containing metaldehyde, another molluscicide, has a recommendation for use as a dilutant at drilling as well as for its molluscicidal properties.

The continued importance of cabbage stem flea beetle as a pest has resulted in recommendations for 10 insecticides (including 3 granular formulations) since 1982 (Table 2). Most can be applied up to plant establishment but several can be applied later if necessary. Seven of these insecticides can also reduce attacks of rape winter stem weevil (Ceutorhynchus picitarsis), a pest which occurs locally in eastern England. Adult feeding damage can occur during plant establishment. Crops emerging before the end of August may require protection, given by insecticides applied at drilling, against cabbage root fly (Delia radicum). All the organophosphates in Table 1 should have been applied as recommended in 1982, after establishment for the control of the summer pest complex.

Potato

The mean number of pesticide treatments received by a potato crop in 1982 was 0.95. The pesticides most frequently applied were the carbamates

(Table 1), of which aldicarb (16061 ha) and oxamyl (16164 ha) (Sly 1984) would have been applied pre-planting, largely for the control of potato cyst nematodes (Globodera pallida and G. rostochiensis). These nematodes are the major pests of potato in the UK, and other temperate parts of the world. Several chemicals are available to the grower to help reduce the damage they cause (Table 3).

Whitehead (1975) estimated the annual loss caused by potato cyst nematodes in England at about £10 million. Use of oximecarbamates can give considerable yield responses (for example, Cutting 1980, Whitehead et al 1980, McKenna and Winslow 1975). The soil fumigants dichloropropene and metham-sodium usually applied in the autumn before planting figured only slighly in the 1982 Pesticide Survey, and this probably still reflects the current use of fumigants against potato cyst nematodes and other soil pests. A considerable part of the best potato-growing land in eastern England is not suitable for the use of fumigants because the surface of organic soils cannot be formed into a good seal, by smearing or rolling, to stop the fumigant from escaping.

Table 3

Maincrop potatoes - major pests and recommended treatments at planting or establishment.

Pest	Chemical
Aphids	aldicarb, disulfoton, thiofanox
Potato cyst nematodes	aldicarb, carbofuran, dazomet, dichloropropene, ethoprophos, metham-sodium, oxamyl
Slugs	copper sulphate mixtures
Stubby root nematodes (tobacco rattle virus)	aldicarb, oxamyl, phorate
Wireworms	aldrin, phorate

The non-fumigant nematicides currently available are all granular formulations and, although the nematodes may attack the plants after establishment, the nematicide must be applied at or before planting in order to incorporate it as much as possible into the root zone of the soil. The time of application is determined on physical as much as biological factors.

Nematicides are required for some crops grown on lighter soils to reduce the risk of the 'spraing' symptoms of tobacco rattle virus, which is transmitted by stubby-root nematodes (Trichodorus and Paratrichodorus).

Few surveyed maincrops had been treated with the aphicidal granules disulfoton or thiofanox at planting. Protection against viruses transmitted by aphids is not as important for maincrops as for seed crops (Carden et al 1983), which require continuous protection from crop emergence. Use of these pesticides on seed crops was probably greater in

1982 than that recorded for maincrops. Wireworms remain minor pests in the UK but aldrin is currently retained for their control where necessary. Satisfactory alternatives have not yet been found (ADAS unpublished).

Sugar beet

In 1982 a sugar beet crop received an average of 1.87 treatments, slightly less than an oilseed rape crop but the proportions of pesticide groups used differed considerably for the two crops. Virtually all sugar beet seed was treated with a carbamate seed dressing (Table 1). Methiocarb is applied to the seed mainly to reduce damage from pygmy mangold beetle (Atomaria linearis), a seedling pest. Carbamates including the seed treatment, accounted for 85% of insecticide, nematicide and molluscicide usage (28% on oilseed rape). Organochlorines constituted 52% of usage on oilseed rape but only 9% on sugar beet. With the exception of pirimicarb (26365 ha), the carbamates (predominantly aldicarb) would have been applied at drilling to control seedling pests (Table 4), and to give some incidental early-season control of aphids and virus.

Table 4

Sugar beet - major pests and recommended treatments applied at planting or during establishment.

Pest	Chemicals
Millipedes, springtails) symphylids, wireworms)	aldicarb and gamma-HCH, bendiocarb, benfuracarb, carbofuran, carbosulfan
Pygmy mangold beetle and millipedes	as above or aldicarb, oxamyl
Beet flea beetle, leatherjackets	gamma-HCH
Slugs	metaldehyde, methiocarb copper sulphate mixtures
Beet cyst nematode	none
Stubby-root and needle nematodes	aldicarb, carbofuran, oxamyl, benfuracarb
Peach-potato aphids	aldicarb, thiofanox

Since the changes in seed rate and other husbandry techniques, many growers have used soil-applied insecticides as insurance treatments, often unnecessarily (Dunning and Byford 1979). However, there is no reliable forecasting system for seedling pest damage available to growers, although techniques for some pests have been developed (Brown 1983, Edwards op cit). Insurance treatments are likely to continue for some time in spite of their varying profitability. Accurate forecasting of crop loss due to Docking disorder, caused by needle (Longidorus spp) and stubby-root nematodes, is unlikely in the near future (Cooke 1976) and nematicides will be needed for crops grown on land with a history of the problem. Fumigant nematicides have given good control (Cooke et al 1973), and, although relatively expensive, may be possible alternatives to granular insecticide/nematicides (Dunning and Byford 1979).

Gamma-HCH, applied as a pre-drilling, or post-emergence spray, has the disadvantage of killing aphid predators and increasing the risk of virus yellows (Dunning and Byford 1983). Several studies have shown the generally deleterious effects of gamma-HCH and other soil-applied insecticides on non-target fauna, especially Carabidae, in sugar beet crops (for example, Hossfeld 1976, Gregoire-Wibo 1980, Konig 1983).

FUTURE DEVELOPMENTS AND CONSTRAINTS

A decision on whether or not to apply a pesticide can be influenced by several factors (Short 1982). The use of pesticides as insurance treatments is understandable in the absence of reliable damage forecasting for several pests and when there is little opportunity to apply a curative treatment (as, for example, when pests attack before plant emergence or when there is no suitable pesticide).

Pesticides are still relatively cheap to buy and apply. As examples, using the averge crop price (Anon 1985) and recommended pesticide price (which is often more than the price a grower pays), the minimum extra yields of produce needed to cover the outlay are:- c 1 t/ha for granular insecticides, and 7-10 t/ha for fumigants (but this could be discounted over the complete rotation), on sugar beet; 2-2.5 t/ha for granular nematicides and dichloropropene, and 5-14 t/ha for dazomet on potato; 0.02 t/ha for pyrethroids, 0.1-0.2 t/ha for granular insecticides and 0.06 t/ha for mulluscicides on oilseed rape. Some of these increases are too small to show as significant losses in most trials. The cost of application has also to be covered but can often be split between 2 or 3 pesticides if tank mixes are used. When it is a small part of an essential operation, such as applying granules at drilling, application costs are negligible. A fall in the market price of a commodity, or removal of a subsidy, would reduce the economic attractiveness of insurance treatments, but conversely could also encourage a grower to maximise his production. The pesticide market is a competitive one and large price increases are probably unlikely at present.

The environmental impact of insecticides, molluscicides and nematicides is increasingly being studied and the desire to reduce harmful effects is likely to be a major factor determining future use. For the control of pests during early growth stages this may mean greater use of suitable seed treatments and of less persistent granules, with more emphasis on the development of good forecasting techniques and thresholds for pest damage.

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THE USE OF PYRETHROIDS TO PROTECT PLANTING MATERIAL AGAINST APHID-BORNE VIRUSES

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ABSTRACT

The pyrethroids cypermethrin and PP321 control the spread of tulip breaking virus (TBV) in tulips and potato virus Y (PVY) in potatoes at least as well as mineral oil but, unlike oil, do not diminish crop yields; as little as 7g a.i./ha of PP321 was effective. An electrostatic sprayer deposited more pyrethroid on potato foliage than a hydraulic sprayer and, when used prior to roguing diseased plants, gave better control of the spread of PVY. Fortnightly spraying of a mixture of cypermethrin and oil diminished spread of PVY by 85% in a seed potato crop.

INTRODUCTION

Planting material is often a source of aphid-borne viruses in crops propagated vegetatively, such as bulbs and potatoes, and some viruses, for example lettuce mosaic virus in lettuce (Tomlinson 1970), are also transmitted through seed. Therefore, it is important that planting material comes from virus-free parents. Systemic aphicides such as those in the organophosphorus and carbamate groups do not kill quickly enough to prevent viruliferous aphids infecting treated plants, inoculation occurring whilst a lethal dose of contaminated sap is being ingested (Schepers 1972). This is especially true for viruses transmitted in the non-persistent manner as these can be acquired and inoculated within minutes.

Synthetic pyrethroids are widely used in European cereal crops to control the persistently transmitted barley yellow dwarf virus and, in experiments, have controlled transmission of several non-persistently transmitted viruses. These include: potato virus M (Lodochkin 1983) and potato virus Y (Gibson 1983) in potatoes, cucumber mosaic virus in cucumbers, maize dwarf mosaic virus in maize (Raccah et al. 1983), tulip breaking virus in lilies and hyacinth mosaic virus in hyacinth (Asjes 1981). The efficacy of pyrethroids appears to be due to particularly fast intoxication of vectors, preliminary symptoms of which are an inhibition of feeding (Sassen 1983) and induction of flight (Gibson 1983).

In this paper we compare the effectiveness of the pyrethroids permethrin, cypermethrin and PP321 (Jutsum et al. 1984) in protecting tulips against tulip breaking virus (TBV) and potatoes against potato virus Y (PVY).

Both viruses are transmitted by aphids in the non-persistent manner and mineral oil sprays provided a standard control measure (Loebenstein & Raccah 1980), used for comparison in initial experiments. Sprays containing mixtures of mineral oil and pyrethroid give particularly good control in laboratory tests (Gibson & Cayley 1984) and field experiments testing their use, integrated with control measures based on husbandry are also described.

MATERIALS AND METHODS

The ability of different pyrethroids to control TBV in tulips

On 19 October 1982, 32 plots, each 1 x 1.7m, were planted with 4 rows of c. 40 tulip bulbs cv Halcro using a stock with c. 10% of bulbs infected with TBV. Sides and ends of plot were separated by 1m-wide fallow paths. Plots were sprayed at high volume (500 l/ha) 7 times at weekly intervals from 5 May to 20 June with the pyrethroids permethrin (50 or 100g a.i./ha), cypermethrin (30 or 45g a.i./ha) or PP321 (30g a.i./ha), with the mineral oil Albolineum AK (15 l/ha) or with the carbamate pirimicarb (250g a.i./ha). Treatments, including an unsprayed control area, were assigned to plots in a randomised block design replicated 4 times. All bulbs from each plot were grown on during the following season and assessed for flower breaking.

The ability of different pyrethroids to control PVY in potatoes

On 21 April 1984, 28 plots, each 4 x 7.5m, were planted with 5 rows of 30 tubers of potatoes cv Bintje, using a potato stock with c. 0.4% of tubers infected with PVY. Sides and ends of plots were separated by unplanted 0.8m-wide paths. Plots were sprayed at high volume (500 l/ha) 9 times at weekly intervals from 1 June (crop emergence) to 24 July with either permethrin (100g a.i./ha), cypermethrin (45g a.i./ha), PP321 (10, 20 or 30g a.i./ha) or Albolineum AK (15 l/ha). Treatments, including an unsprayed control area, were assigned to plots in a randomised block design replicated 4 times. A sample of 100 tubers from each plot was grown on and the foliage assessed visually for symptoms of infection.

Use of pyrethroids and oil to aid roguing potatoes

On 16 April 1984, 32 plots, each 3.8 x 10m, were planted with 5 rows of potatoes cv King Edward using 25 virus-free potatoes to plant each of the outer two rows and 25 PVY-infected tubers to plant the central row. Plot ends were separated by 6m planted with potatoes cv Desiree and plot sides by two fallow 1.5m paths on either side of a central strip 2.3m wide of potatoes cv Pentland Crown. Both Desiree and Pentland Crown are virus-resistant. Plots were sprayed either hydraulically (200 l/ha) or with electrostatically-charged rotary atomizers (Arnold 1983) at 8.9 l/ha on 23 and 31 May with either cypermethrin (40g a.i./ha), Sunoco 7E mineral oil (3 l/ha) or a mixture of both cypermethrin and Sunoco 7E, or were not sprayed; the untreated plots were replicated 8 times whilst treated plots were replicated 4 times. On 8 June all PVY-infectors were dug up and removed from all plots. Any of the initially healthy plants which developed symptoms of PVY were recorded and also rogued.

In 1985, the experiment was repeated and plots were planted on 17 April and sprayed on 29 May and 5 June hydraulically or electrostatically with either cypermethrin (40g a.i./ha), PP321 (7g a.i./ha), Sunoco 7E (7 l/ha) or with mixtures of cypermethrin and Sunoco 7E or of PP321 and Sunoco 7E. Infectors were rogued on 12 June and subsequently infected plants when symptoms were seen. Deposits of pyrethroids on leaves were analysed as described by Gibson & Cayley (1984).

Production of once-grown seed potatoes using pyrethroids and oil

On 10 April 1984, 16 plots 18 x 6m were planted with 24 rows of 25 potatoes (FS seed) either of cvs King Edward or Maris Piper. There was no separation between plot sides but each plot end was planted with 4m of FS potatoes cv Desiree and separated by a further unplanted 6m strip. The entire experiment was treated at planting time with granules of phorate (1.7kg a.i./ha) and fortnightly with pirimicarb (140g a.i./ha) sprays applied hydraulically from 15 June to 13 August. 8 plots were also sprayed from full plant emergence (31 May) with seven fortnightly sprays of a mixture of cypermethrin (40g a.i./ha) and SC 811 mineral oil (7 l/ha). A sample of 160 tubers from each plot was grown on and the foliage checked for virus symptoms.

Statistical analyses

Results were either arcsin- or logit-transformed before an analysis of variance.

RESULTS

The ability of different pyrethroids to control TBV in tulips

TABLE 1

Effect of different sprays on the spread of TBV and on bulb yield

Spray	Rate/ha	New infections %	New infections Transformed*	Bulb wt (kg) harvested/plot
PP321	30g a.i./ha	4.3	9.7	7.12
Permethrin	50g a.i./ha	22.0	28.6	6.76
Permethrin	100g a.i./ha	15.3	23.7	6.74
Cypermethrin	30g a.i./ha	12.0	20.9	6.60
Cypermethrin	45g a.i./ha	7.5	11.7	6.82
Mineral oil	15 l/ha	8.3	13.9	6.48
Pirimicarb	250g a.i./ha	15.8	25.2	6.43
Untreated	-	16.0	24.2	6.47
Standard error of means			3.4	0.27

* Arcsin-transformed and weighted for number of initially-infected bulbs/plot.

Both PP321 and the higher rate of cypermethrin more than halved the spread of TBV, controlling it at least as well as mineral oil; permethrin and pirimicarb gave no apparent control. Plots treated with PP321 and cypermethrin (45g a.i./ha) yielded most, but there were no significant ($P > 0.05$) increases in yield due to spraying.

The ability of different pyrethroids to control PVY in potatoes

TABLE 2

Effect of different sprays on the spread of PVY and on tuber yield

Spray	Rate/ha	Tubers with PVY (% arcsin-transformed)	Tuber yield ($kg/18m^2$)
PP321	10g a.i./ha	11.1	46.2
PP321	20g a.i./ha	6.1	47.3
PP321	30g a.i./ha	12.3	48.2
Permethrin	100g a.i./ha	6.1	45.5
Cypermethrin	45g a.i./ha	10.0	47.0
Mineral oil	15 l/ha	10.6	42.1
Untreated	-	19.8	48.4
Standard error of means		3.0	1.0

The pyrethroids and the oil all approximately halved the spread of PVY. None of the pyrethroids increased yield but oil sprays decreased it.

Use of pyrethroids and oil to aid roguing potatoes

TABLE 3

Percentage of new infections of PVY in plots treated with oil and pyrethroids before roguing.

Spraying Method	Year	Cypermethrin	PP321	Oil	Cypermethrin + Oil	PP321 + Oil	Untreated
Electrostatic	1984	3z*	-	8y	3z	-	17y
	1985	2a	3ab	3ab	3ab	1a	8c
Hydraulic	1984	4zy	-	11y	5z	-	17y
	1985	2ab	5b	5ab	2a	2ab	8c

* Means within a year with no letter in common are significantly ($P < 0.05$) different

Treating plots with cypermethrin or PP321 before roguing decreased spread of PVY but oil decreased spread significantly ($P < 0.05$) only in 1985 when it was applied at 7 l/ha. Plots sprayed with mixtures of oil and PP321 had fewer infected plants than plots sprayed with either oil or PP321 alone, but this was not a significant difference. The foliage of plots sprayed electrostatically with PP321 received 16.5 ± 2.3mg/kg dry leaf and with cypermethrin 96.2 ± 2.9mg/kg whereas those sprayed hydraulically received only 5.2 ± 0.7mg/kg and 38.8 ± 1.2mg/kg respectively; sprays applied electrostatically generally gave the better control.

Production of once-grown seed potatoes using pyrethroids and oil

TABLE 4

Spread of PVY and tuber yield in a seed potato crop

	Tubers with PVY		Tuber yield (t/ha)
Systemic aphicides plus cypermethrin and oil	%	Logit	
cv King Edward	1.25	-2.14	25.3
cv Maris Piper	0.94	-2.25	23.8
Systemic aphicides alone			
cv King Edward	9.22	-1.37	33.8
cv Maris Piper	5.31	-1.69	32.3
Standard error of difference		0.21	2.7

No plants of either King Edward or Maris Piper initially had symptoms of PVY. However, infection was introduced, probably from groundkeepers and adjacent crops and when grown on, the proportion of tubers infected with PVY in plots sprayed regularly with the cypermethrin : oil mixture was one seventh that in plots receiving only systemic aphicides.

The cypermethrin : oil mixture was phytotoxic and treated plots yielded about 25% less than untreated plots.

DISCUSSION

The results indicate that cypermethrin and PP321 controlled spread of TBV and PVY at least as well as Albolineum AK mineral oil, and had an advantage over the oil in not diminishing yield through phytotoxicity. As reported elsewhere (Perrin & Gibson 1985), as little as 7g a.i./ha of PP321 was effective and greater application rates gave no apparent improvement, so even 7g a.i./ha may be excessive. Use of an electrostatic sprayer, which increased chemical deposition on plants threefold, may enable rates to be decreased even further.

Freedom of planting material from aphid-borne viruses is at present maintained largely by crop hygiene and avoiding vectors. Pyrethroids may allow such restrictions to be relaxed enabling virus-free crops of, for example, potato to be grown where it was previously impossible. Pyrethroids may also be used as an additional protective measure, integrated within conventional systems for controlling aphid-borne viruses. Thus, treatment contained virus spread prior to roguing and would clearly be beneficial if it is either inconvenient or difficult to rogue, as in newly-emerged potatoes when virus symptoms are indistinct, and in tulips before they flower.

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CEREAL SEED TREATMENT WITH FLUTRIAFOL-BASED MIXTURES

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ABSTRACT

Flutriafol at 50-75ppm, in mixture with other co-fungicides, controls all the major seed-borne diseases of wheat and barley. At 150ppm, the compound, in mixture with other co-fungicides including ethirimol at 2000ppm, gives excellent dual mode-of action control of barley powdery mildew (Erysiphe graminis hordei). Examples from recent trials demonstrate the value of flutriafol-based mixtures in achieving healthy seeds and crops of temperate cereals.

INTRODUCTION

Most of the 700,000 tonnes of cereal grain used as seed in the UK is treated with a fungicide. Broad-spectrum systemic seed treatments which give control of early foliar diseases as well as seed and soil-borne diseases account for about 30% of the market while almost all of the remainder is made up of organomercurial compounds. As mercury has not been banned in the UK only small amounts of the more expensive mercury replacement formulations are used.

Fungicidal seed treatments are used fo fulfil one or more functions:-

1. To destroy pathogens on seed surfaces (eg, wheat bunt caused by Tilletia caries).
2. To eradicate deep-seated and embryo-borne infections (eg, loose smut of barley caused by Ustilago nuda).
3. To protect the emerging seedling from soil-borne fungi (eg, damping-off caused by Fusarium spp. and Pythium spp.).
4. To protect the young plant from air-borne infections (eg, powdery mildew caused by Erysiphe graminis).

The principal fungal pathogens of wheat and barley which may be controlled by seed treatments in the UK and Western Europe include Ustilago hordei (covered smut of barley), Ustilago nuda (loose smut of barley), Ustilago tritici (loose smut of wheat), Tilletia caries (bunt of wheat), Pyrenophora graminea (leaf stripe of barley), Pyrenophora teres

(net blotch of barley), Septoria nodorum (seedling blight of wheat), Fusarium spp. (seedling blights), Fusarium nivale (snow mould), Rhynchosporium secalis (leaf blotch of barley) and Erysiphe graminis (powdery mildew).

The organic fungicides used as alternatives to organomercurials vary in their properties. Thiram and captan have useful action against soil and seed pathogens but do not control embryo-borne infections such as loose smut of wheat and barley. More recently introduced compounds used in mixture provide good control of the major seed and soil-borne diseases including loose smut. These compounds include carboxin, fenfuram and methfuroxam (oxathiins), fuberidazole, thiabendazole and thiophanate methyl (benzimidazoles), guazatine and the ergosterol biosynthesis inhibitors (EBIs), bitertanol and imazalil.

In addition, some compounds are used to provide systemic control, early in the season, of air-borne infections (especially powdery mildew) via seed treatments. These include ethirimol (a hydroxypyrimidine) and the EBI fungicides such as triadimenol and flutriafol. The activity of flutriafol was described by Skidmore et al (1983). Ethirimol has a narrow spectrum of action against powdery mildews only, while the EBI compounds also are active against early infections of rusts (Puccinia spp.), Rhynchosporium secalis and Pyrenophora teres. A mixture containing flutriafol and ethirimol was announced by Northwood et al in 1984. This mixture was shown to give mildew control and yield increases superior to current commercial treatments as well as offering good control of seed and soil-borne diseases.

This paper describes recent laboratory and field studies which have evaluated a range of formulations containing flutriafol compared with other commercial standards. It highlights the advances achieved in terms of rate reduction, efficacy, spectrum and crop safety with these mixtures.

MATERIALS AND METHODS

The formulations containing flutriafol which were tested during 1984/1985 on winter and spring barley and winter wheat are listed in Table 1, along with the commercial standards used.

The standard MAFF germination test was used to assess the safety of formulations to a wide range of wheat and barley cultivars. Four replicates, each of 100 seeds, were sown in Levington Universal compost at a depth of 15mm in seed trays, watered, covered and kept at a constant temperature of 20°C for 6 days. Tests were assessed by a qualified seed analyst and the percentage of normal seedlings recorded.

TABLE 1

Formulations tested during 1984/1985 season.

(a) Formulations containing flutriafol.

Formulation Code	Active Ingredients	Type of Formulation*	Dose - ppm on seed
1	Flutriafol/thiabendazole/ethirimol - 'Ferrax'	FS	150/50/2000
2	Flutriafol/thiabendazole/ethirimol/ imazalil	FS	150/50/2000/30
3	Flutriafol/thiabendazole/imazalil	LS	75/50/30
4	Flutriafol/thiabendazole	LS	50/50
5	Flutriafol/thiabendazole	PS	50/50

(b) Commercial standards used.

Formulation code	Active Ingredients	Type of Formulation*	Dose - ppm on seed
A	Carboxin/thiabendazole/imazalil - 'Cerevax' Extra	LS	900/75/60
B	Carboxin/thiabendazole - 'Cerevax'	LS	600/50
C	Phenyl mercury acetate - 'Ceresol'	LS	22
D	Triadimenol/fuberidazole - 'Baytan'	PS	375 + 45
E	Triadimenol/fuberidazole/imazalil - 'Baytan IM'	PS	375 + 45 + 50

*FS = flowable suspension; LS = liquid; PS = powder

All seed and soil-borne disease trials used naturally infected seed stocks, and were fully replicated using randomised block designs and were conducted at Jealott's Hill Research Station and at an experimental site at Mildenhall, Suffolk. Seed was treated in a laboratory-scale Rotostat machine (Harris, 1975). Plot sizes were 1m x 3m and were drilled using a Wintersteiger Seedmatic 6 small plot drill.

Four unreplicated powdery mildew trials were undertaken on Triumph spring barley at locations in East Anglia. Seed was treated in a commercial Rotostat machine and drilled into plots of 120m x 40m approximately. Treatments were randomised in adjacent strips.

For all trials, plant emergence, height and disease assessments were made according to recognised guidelines on 10 to 25 tiller, plant, leaf or ear samples per plot. Duncan's multiple range test (Duncan, 1955) was used to compare statistically each treatment mean and values followed by a common letter are not significantly different at $P = 0.05$.

RESULTS

Seed germination figures from growth room crop safety studies with formulation 1 are presented in Table 2 for a range of winter and spring barley cultivars. The formulation was safe, giving greater than the minimum 85% normal germination required by the Cereal Seed Regulations, 1980.

Crop emergence figures from a field trial drilled late in the season to exacerbate crop stress are presented in Table 3 for Aquila winter wheat. All EBI-based formulations had a tendency to cause a slight reduction in speed of emergence but eventual crop establishment was good.

Control of Tilletia caries is demonstrated in Table 4. The formulation containing flutriafol gave complete control of wheat bunt whereas the mercury standard gave incomplete eradication. Table 4 also presents results against Fusarium nivale infected wheat seed. Control of the pathogen was manifested as improved crop emergence compared to untreated seed.

For control of winter barley leaf stripe, two flutriafol-based formulations containing imazalil were evaluated. Table 4 indicates the high levels of control that were obtained with these formulations. Formulation 1 (containing flutriafol/ethirimol/thiabendazole) gave good control of the disease and this was further improved with the addition of imazalil to the mixture (formulation 2). A range of flutriafol-based formulations gave excellent control of Ustilago nuda, where mercury treatment was ineffective. The carboxin standard also gave good control of the disease.

TABLE 2

Percent germination of a range of winter and spring barley cultivars in growth room tests, following treatment with a flutriafol/ethirimol/ thiabendazole formulation.

Formulation	WINTER BARLEYS				SPRING BARLEYS			
	Gerbel	Igri	Sonja	Pirate	Kym	Atem	Patty	Carnival
1	99	93	94	97	94	93	90	90
D	98	92	97	98	96	94	89	92
E	98	95	86	99	95	95	nt	nt
Untreated	98	98	93	98	94	98	93	92

nt = not tested

TABLE 3

Crop emergence in Aquila winter wheat following treatment with two flutriafol-based mixtures, UK, 1984. Expressed as percentages of untreated control.

Formulation	% of Plants Emerged/Metre	
	17 DAS*	23 DAS*
4	95	93
5	94	92
B	93	88
D	93	93
Untreated (actual)	100 (55.2)	100 (57.2)

DAS* = days after sowing

TABLE 4

Percent control of seed-borne pathogens of winter wheat and winter barley with flutriafol mixtures, UK, 1984/1985.

Formulation	WHEAT		BARLEY	
	Tilletia caries cv. Hustler GS 70	*Fusarium nivale*# cv. Dolmit 44 DAS*	*Ustilago nuda* cv. Panda GS 60	*Pyrenophora graminea* cv. Not known 210 DAS*
1	nt	nt	99.9 a	68 a
2	nt	nt	100.0 a	98 b
3	nt	171 a	100.0 a	98 b
4	100 a	169 a	99.8 a	
A			99.1 a	98 b
B	100 a	171 a		
C	82 b	180 a	24.3 b	99 b
D	100 a	167 a	100.0 a	
E				96 b
Actual disease level on untreated	(65.4) c	(48.2) b	(15.4%) c	(6.4%) c

nt = not tested

*DAS = Days after sowing

Values in columns followed by a common letter are not significantly different at P = 0.05

Values are percentage increases in numbers of plants emerged compared to untreated control

Superior performance against powdery mildew of the formulation containing flutriafol and ethirimol over the triadimenol mixture was seen in 1985 trials with spring barley (see Table 5).

TABLE 5

Percent powdery mildew 70 days after drilling on Triumph spring barley treated with flutriafol mixture formulation 1 at four locations in East Anglia, UK, 1984/1985. Leaf 3 was assessed.

Trial Location	Treatment 1	Treatment D	Not treated
Stow cum Quay	0.7	6.7	6.5
Hepworth	0.8	2.9	6.2
Colne	5.1	6.3	13.6
Wattisham	2.5	10.1	10.8
Means of above trials	2.3	6.5	9.3

DISCUSSION

The results illustrate several features of a modern chemical approach to the control of seed, soil and air-borne diseases affecting plant health. Flutriafol has broad-spectrum action at low rates against the major seed-borne pathogens. It is systemic and eradicates embryo-borne pathogens and has a persistence of effect which gives long-term protection against early air-borne infections of foliar pathogens. Used in mixture with ethirimol, particularly for control of powdery mildew, it has proven superior to a formulation based on a single mildew component. In seven further fully replicated trials on winter and spring barley during 1984/1985 this mixture was superior to the standard (Northwood et al, 1985). The lower efficacy of triazole fungicides has been related to the development of decreased sensitivity in powdery mildew populations (Fletcher and Wolfe, 1981; Heaney et al, 1984). It is hoped that by mixing chemicals with differing modes of action (such as flutriafol and ethirimol) the development of fungicide resistance will be avoided or delayed.

The effects of failure to control high levels of seed-borne infections can be devastating while the use of seed treatments for control of early air-borne infections can have significant benefits to the farmer in terms of easier crop management, improved yields and higher grain quality.

The yield benefits of using flutriafol-based seed treatments have been reported elsewhere (Skidmore et al, 1983; Northwood et al, 1984,1985). Other features include broad spectrum of action, high levels of disease control and increased environmental safety compared to organomercurial products. They allow the farmer to attain high levels of crop health at the time of germination and crop establishment and form a major part of the modern integrated approach to crop management.

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USE OF FUNGICIDE AND PESTICIDE SEED TREATMENTS TO ENHANCE RYEGRASS ESTABLISHMENT

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ABSTRACT

Fungicide and pesticide treatments for ryegrass seed were studied in a series of pot, micro-plot and small plot experiments at Hurley. Benomyl + captan was the most effective of the various fungicide seed treatments tested. Perennial ryegrass seed with or without treatment with benomyl + captan was sown at monthly intervals from April to October 1984 and seedling emergence was improved by fungicide treatment at four sowings, when seedbed conditions were warm and dry.

Seed treatment of Italian ryegrass with fonofos increased tiller population and herbage yield by controlling damage by stem-boring larvae of the frit-fly complex.

A combination of seed treatment with benomyl + captan and post-emergence sprays of chlorpyrifos and omethoate markedly improved ryegrass establishment. A combined seed treatment however would offer a cheap and simple method for improving ryegrass establishment, with low risk to the environment. Preliminary work using a combination of benomyl + captan and fonofos seed treatments showed no benefits, but further work is in progress.

INTRODUCTION

Spores and other propagules of Fusarium, the most important genus of seedling diseases on ryegrass (Lolium) (Holmes 1979) are prevalent in soils throughout the UK (McKenzie & Taylor 1983). Warm or dry conditions predispose seedlings to attack by these fungi (Lewis 1985) and unless seeds are protected while such conditions prevail, a high level of pre-emergence seedling mortality may occur. The fungicides benomyl and captan have been shown to greatly improve grass seedling emergence (Holmes, 1983; Lewis, 1985).

Little plant material is present during the establishment phase and hence a low incidence of pests can cause significant damage to a newly-sown ryegrass sward. The most important pests of autumn sown grass are stem-boring larvae of the frit fly complex (e.g. Oscinella frit) and unless seeds or seedlings are treated great damage may be caused by these larvae, especially to swards sown in late August (Clements & Bentley 1985).

The present work involved the examination of fonofos seed treatment for the control of frit fly and a range of fungicide

seed treatments for the control of seedling diseases, with the aim of assessing the potential of a combined fungicide and pesticide seed treatment.

MATERIALS AND METHODS

The field experiments were carried out at Hurley and soil from the field, which was of a sandy type, was used in the pot experiment. For the field experiments, fertilizer was applied to the seedbed at a rate of 80, 40, 40 kg/ha of N, P_2O_5 and K_2O respectively.

Fungicide and pesticide treatments

The following treatments were used in the experimental work:

(a) benomyl (technical, 95% s.p.; Du Pont Ltd)
(b) captan (Captan 83, 83% w.p.; Murphy Chemical Ltd)
(c) captan (Captan 50F, 50% flowable, Stauffer Chemical Ltd)
(d) carbendazim (Derosal Liquid, 51% e.c., Hoechst UK Ltd)
(e) metalaxyl + thiabendazole (Apron T, 69% w.p., Ciba-Geigy Agrochemicals)
(f) metalaxyl + captan (Apron 70SD, 70% w.p., Ciba-Geigy Agrochemicals)
(g) drazoxolon (Saisan 30, 10% e.c., S.A.I. PLC)
(h) fonofos (Fonofos Seed Treatment, 43% e.c., Stauffer Chemical Ltd)
(i) chlorpyrifos (Dursban 4, 48% e.c., Dow Chemical Co. Ltd.)
(j) omethoate (Folimat, 58% e.c., Bayer UK Ltd)

Dose rates used in the various experiments are given below.

Fungicides in powder formulation were applied by mixing them with the seed in a plastic drum rotated at 60 rev/min for 5 minutes. Fungicides in liquid formulation, and fonofos were applied in a mini-Rotostat.

Foliar sprays of pesticides were applied by a wheeled plot-sprayer constructed at the Animal and Grassland Research Institute and using compressed air to propel the liquid. The chemicals were applied in 400 litres water/ha, using a spray pressure of 140 KPa and a forward speed (hand-powered) of 0.4 m/s. The nozzles used were Tee-jets from Spraying Systems Co. with 80015 LP tips.

Expt 1. Pot test

Plastic pots of 14 cm diameter were filled to within 2 cm of the rim with sieved soil and 100 seeds/pot of perennial ryegrass cv. Parcour were broadcast over the surface and covered with soil to a depth of 2 cm. The seed sown was treated with one of four fungicide treatments: benomyl + captan (3g + 3g a.i./kg seed), metalaxyl + thiabendazole (2.8g + 1.5 ga.i./kg seed), captan + fonofos (pesticide) (1.5g + 4 ga.i./kg seed) and metalaxyl + captan (1.4g + 1.4g a.i./kg seed). Five pots were sown with seed of each treatment, and with untreated seed, and the pots were randomised. Each pot was given 100 ml of water after sowing and

the pots were kept in a glasshouse maintained at a temperature of about 20°C. Numbers of seedlings emerging were recorded for each pot.

Expt 2. Micro-plot test 1984

Micro-plots were sown at monthly intervals from April to October 1984, with 100 seeds/plot of perennial ryegrass cv. Parcour, 4 cm apart on a 10 x 10 grid pattern. Seeds were covered with sieved soil to a depth of 2.5 cm. At each sowing one micro-plot was sown with seed treated with benomyl + captan (3g + 3g a.i./kg seed) and one with untreated seed, in each of four replicate blocks. Numbers of seedlings emerging were recorded for each plot 20 to 63 days after sowing. Soil temperature and moisture at seed depth were monitored.

Pesticide seed treatment

Expt 3. Small plot test sown 1982

Small plots, size 6 x 1.5 m, of Italian ryegrass cv. RvP, were drilled at Hurley on 25 August 1982. Plots were treated with one of 11 pesticide treatments which included fonofos seed treatment at a rate of 4 g a.i./kg seed. Plots were laid out in five replicate randomised blocks. Tiller population, herbage yield and the pest population of the developing sward were assessed in autumn 1982, and on five subsequent occasions from May through November in 1983 (Clements et al. 1985).

Expt 4. Small plot test sown 1984.

Experiment 4 was direct-drilled on 10 October 1984 and tested the effect of (i) fonofos spray at 0.9 kg a.i./ha and (ii) fonofos seed treatment on herbage yield in May 1985.

Combined fungicide and pesticide treatment

Expt 5. Small plot test 1984

Plots (size 1.5 m x 6 m) of Italian ryegrass cv. RvP and perennial ryegrass cv.Parcour were drilled at Hurley on 8 August 1984, using a seed-rate of 1000 viable seeds/m^2. Three treatments were applied to each of the two cultivars: fungicide seed treatment using benomyl + captan (3g + 3g a.i./kg seed); insecticide sprays using chlorpyrifos at 720 g a.i./ha at early emergence followed by omethoate at 650 g a.i./ha at full emergence; seed treatment plus spray as above.

The treatments were applied to plots of each cultivar arranged at random in each of five replicate blocks. An untreated plot of each cultivar was included in each block.

Seedling emergence, tiller populations and herbage yield were assessed in autumn 1984 (Lewis & Clements, 1985).

Expt 6. Small plot test 1985

Plots (size 1.5 m x 6 m) of perennial ryegrass cv. Parcour were drilled on 3 July 1985 using a seed-rate of 1000 viable seeds/m^2. The seed was treated with five fungicide and/or

pesticide treatments: fonofos (4g a.i./kg seed), carbendazim + captan (1.5g + 1.5 g a.i./kg seed), fonofos + carbendazim + captan (same rates as above), benomyl + captan (3g + 3g a.i./kg seed), and drazoxolon (3g a.i./kg seed). The treatments were applied to plots arranged at random in each of five replicate blocks. Two untreated plots were included in each block. Plots sown with seed treated with benomyl + captan were sprayed with chlorpyrifos and omethoate as for Expt 5.

Seedling emergence and herbage yield were assessed (see Table 5 for dates).

RESULTS

Expt. 1. Pot test: Effect of four fungicide treatments on emergence of perennial ryegrass.

Seedling emergence was increased by seed treatment with benomyl + captan and metalaxyl + thiabendazole (Table 1).

TABLE 1

Emergence (%) of perennial ryegrass seedlings after sowing untreated seed and seed treated with four fungicide combinations.

Treatment	
benomyl + captan	96.6**
metalaxyl + thiabendazole	92.6**
fonofos + captan	86.0
metalaxyl + captan	84.2
Nil	80.2
SED (DF 16)	4.19

** significant increase over nil treatment ($P<0.01$)

Expt 2. Micro-plot test 1984: Effect of fungicide seed treatment on emergence of perennial ryegrass at seven sowings.

Seed treatment with benomyl + captan significantly increased emergence at four of the seven sowings, and these four sowings were associated with periods of warm, dry soil conditions (Table 2). Heavy rain fell during August but the soil quickly dried out afterwards, due to the high soil temperatures.

TABLE 2

Date of sowing in 1984	% increase in seedling emergence.	Seed-bed conditions for 20 days after sowing: Mean max. soil temp. (°C) at 2.5 cm	Mean % soil moisture
5 April	-0.7	13.0	9.6
2 May	20.6**	14.5	7.7
6 June	28.0**	24.5	8.5
2 July	20.8**	25.5	3.5
1 August	16.2*	25.8	13.4
3 September	2.0	16.8	10.6
8 October	9.4	11.2	16.0

** significant increase over untreated seed (P<0.01)
* significant increase over untreated seed (P<0.05)

Pesticide seed treatment

Expt 3. Small plot test sown 1982

All treatments significantly increased tiller population and/or herbage yield in November 1982 and/or for the whole of the following year. Stem-boring larvae were the major pests present. Yield enhancement attributable to fonofos seed treatment (2.5 t/ha) was nearly as great as that by any other treatment and was achieved at much less cost (around £6/ha) (Clements et al. 1985).

Expt 4. Small plot test sown 1985

Yields were variable, but responses to the fonofos seed treatment and fonofos spray treatment in May 1985 were 0.45 and 0.82 t dry matter/ha respectively.

Combined fungicide and pesticide treatment

Expt 5. Small plot test: Effect of fungicide seed treatment and post-emergence pesticide sprays on ryegrass establishment.

Results

The combined fungicide and insecticide treatment increased tiller population and herbage yield on both cultivars. The tiller population and herbage yield of RvP, but not of Parcour were increased by the insecticide treatment alone. The fungicide treatment alone had no effect on either cultivar (Lewis & Clements, 1985).

Expt 6. Small plot test 1985: Effect of fungicide and pesticide treatment on perennial ryegrass establishment

Benomyl + captan increased seedling emergence and, in conjunction with the post-emergence pesticide sprays, increased herbage yield (the sprays were applied after the seedling emergence assessment). Carbendazim + captan also increased herbage yield (Table 3). Combined insecticide and fungicide seed treatment showed no benefits in this test.

TABLE 3

Effect of fungicide and pesticide treatments on establishment of perennial ryegrass.

Treatment	% increase over untreated seed	
	seedling emergence (22.7.85)	herbage yield (6.9.85)
Fonofos	6.7	2.6
Carbendazim + captan	9.6	18.4*
Fonofos + carbendazim + captan	-6.0	5.2
Benomyl + captan + chlorpyrifos + omethoate	23.7*	27.2**
Drazoxolon	3.1	7.7

* significant increase over untreated seed ($P<0.05$)
** significant increase over untreated seed ($P<0.01$)

DISCUSSION AND CONCLUSIONS

The effects of dry and warm seedbed conditions in predisposing perennial ryegrass seedlings to attack by soil-borne diseases were confirmed. Benomyl + captan was the most effective fungicide treatment tested and is very economical - around £0.04/kg seed.

The current work showed the potential for fonofos as a seed treatment to control frit fly and to enhance markedly the yield of autumn sown Italian grass. Further work by Mathews, Cottey & Clements (in press) has confirmed these early observations and shown there is little or no danger to wildlife from using fonofos seed treatment on a field scale. This product has now been launched on the UK market and costs around £0.22/kg seed.

A combined fungicide and pesticide seed treatment might offer a simple and cost-effective means of guarding against potential losses from soil-borne diseases and frit fly attack. Also, there would be less risk to the environment than from pesticide sprays. In the present work, improved establishment was obtained only when fungicide seed-treatment was used alone or with pesticide sprays, whereas a combination of fonofos and fungicide as a seed treatment had no effect. However, this latter combination was tested in July, when significant damage by frit fly is unlikely to occur, and further sowings will be made in autumn 1985, during the period when losses from frit fly attack are greatest.

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6.
Technologies

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INTRODUCTION TO THE USE OF MICROPROPAGATION IN CLEAN STOCK PROGRAMMES

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Micropropagation, based on tissue culture techniques, is the swiftest method available at present for multiplication of high grade stocks of crop plants. Several multiplication systems now incorporate a micropropagation stage and others are under study. Few have gone beyond the substitution of an existing stage by micropropagation, onwards to the design of the entire system to make use of its powerful advantages.

The difficulties and problems of maintaining a high health status and of making available stocks of guaranteed varietal purity for vegetatively propagated crops are by now well known to all who are involved with crops. Adequate reviews of the extensive literature on this subject are to be found in papers, such as Ebbels (1979a and b) and others dealing with specific crops.

Whenever conditions and costs warrant, schemes have been evolved which rely on the establishment and maintenance of a small nucleus of true-to-type plants guaranteed to be free from a list of pathogenic organisms for which testing facilities exist. At present these plants are maintained in quarantine conditions and repeatedly or annually tested before being used for the generation of definitive or classified stocks.

Tissue culture systems for medium term storage of germplasm by use of temperatures between 3° and 9°C or with the aid of growth retardants (Damiano 1979, G. Mix personal communication) is a well established technique not yet put to use in this field. It is possible to store for between two and seven years with few transfers to fresh medium.

In many countries schemes now exist to regulate the multiplication phase which follows, and this is most often linked to inspection and certification arrangements. The degree of state participation, grower control, the intensity of inspection procedures, and the detail contained in the legislation vary between countries and schemes. Britain possesses a good range of these with bulb and potato illustrating simplicity and complexity, respectively. Stocks of bulbs, for instance, are controlled by the Nuclear Stock Association (Bulbs) composed of substantial growers. The chosen varieties are lodged with the Glasshouse Crops Research Institute, who clean, test and hold them against a call for distribution to growers. In Scotland the same function was performed by the Scottish Crop Research Institute and the College of Agriculture. From time to time fresh stocks are released to growers after a multiplication stage inspected by, in the latter case, staff of the Department of Agriculture. Such stocks may be used for many years before a new one is asked for. Until there is a steady flow of new varieties there is little likelihood of much expansion.

In contrast to the simplicity of this arrangement for multiplication of Narcissus, in Scotland and Northern Ireland the potato certification scheme has a long, expensive chain of field multiplication between the nuclear stocks and the commercial grower, every step governed by detailed legislation and monitored by careful inspection. Few other countries have adopted systems of this complexity and intensity. It has grown over a period of 66 years, been changed and added to, but not reviewed as a whole.

It is the stage of field multiplication from nuclear stock to commercial quantity which is the weakest part of the process. Thirty-five million strawberry runners are planted annually in the United Kingdom, all generated from approximately 100 original plants. The stocks of bulbs are raised from as few as five clean, small bulbs.

In a mass crop such as the potato, planting densities are between 25 and 40 thousand tubers per hectare, which means that the increase from the few healthy plants maintained by the Department of Agriculture and Fisheries for Scotland (DAFS) is so staggeringly large as to make it reasonable to question whether the original premises of the scheme are sound. However, complete eradication of all pathogenic organisms is not the purpose of the scheme. A continual input of clean plants is assumed to push away the main sources of reinfection, thus allowing stocks a longer life before degeneration appears, and the scheme has succeeded in this.

Until very recently the multiplication phase was limited by the rates of conventional increase, which lie between X2 for bulbs and X10 for potatoes. Enhanced propagation rates have been achieved by forcing potato stems under glass for production of cuttings and by chipping or twin scale dissection of bulbs. These techniques have helped to shorten the time taken to acquire sufficiently large numbers of plants.

Micropropagation would seem to provide an even faster and more economical means of increase than these as its main use is in the generation of numbers of the order of tens or hundreds of thousands up to one to two million within a space of nine to twenty months. The slow progress in the introduction of the technique can partly be explained by a bad start from a well known disaster with the strawberry Domanil, which Dr. Boxus, unjustly, is not allowed to forget, some suspected errors in labelling or inadequate inspection in Canadian crops, and the association of callus and cell culture as means of generating variation for plant breeders. All these combined to throw suspicion on the capacity of the method of maintaining varietal purity. Now, some ten years later, it can be stated unequivocally that there is very little chance of the process itself generating more or different variations, aberration or mutations than any other propagation system.

Micropropagation is used very successfully in Scotland for the production of up to 9,000 strawberry runners from nuclear stock material provided by East Malling Research Station. Two years of field multiplication bring numbers up to the three to five million required by the country each year (Harper, Fordyce and Rankin 1985). Experience over five years with twelve varieties suggest that the inspection/multiplication phase can be reduced to one year with considerable benefit to growers and some savings in costs.

For virus indexed Narcissus bulbs it has proven possible to generate upwards of 2,500 bulbs over fifteen months, and, if followed by chipping, a stock can be had in four to five years as large as that which formerly took over fifteen years - some 100,000.

Definitive stocks of potatoes, prior to classification, are now micropropagated by the DAFS. Production of between twelve and thirty thousand plantlets annually cuts out some of the field and glasshouse multiplication stages undertaken by the State before tubers are released to growers of VT grade tubers. So far only two growers have been licenced to generate virus

tested stocks by micropropagation, producing near to 50,000 plantlets between them. There is still a six to seven year gap from release to commercial growers during which time reinfection occurs. Rates of failure and downgrading vary from year to year, and, occasionally, low quality material arrives in the hands of the buyer, giving opportunities for complaints and sometimes for legal action.

In the United States both micropropagated plantlets and tubers derived from them in pots under glass are on sale to private growers. The plantlets are offered for 33 to 50 cents, minimum order 1,000, and the tubers at 40 to 50 cents with a minimum order of 20,000, enough to plant an acre.

These prices may seem surprising to British growers used to being given nuclear stock material for 50p to £1 per tuber or buying VT (SC) grade at 6p or less per tuber. It does demonstrate that in the absence of a heavily subsidised State produced article, the grower will pay a reasonable price. In the strawberry crop, Scottish runner producers pay ten times the commercial rate of foundation grade runners. This would seem to close the argument that microplants are too expensive for all but the earliest stages of the process.

The success of the Scottish strawberry certification scheme demonstrates the benefits to be derived from making full use of a technology which is no longer new. The potato certification scheme has become too rigid to be able to use the technique in a way which will exploit its full advantages. This must be surprising to outsiders who see the potato crop as producing a low cost article intended for mass consumption; not at all the kind of crop which merits an elaborate and expensive inspection scheme. The proper use of tissue culture techniques will simplify the process and put more of its control into the hands of the producers, leaving the State free to return to its role as inspector and mediator.

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VIRUS DETECTION BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) IN THE PRODUCTION OF HEALTHY PLANTS

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ABSTRACT

Virus detection in vegetatively propagated plants demands techniques which are sensitive, reproducible, which do not need high levels of skill or labour input and which are reasonably rapid. The range of such techniques available is limited. Traditional transmission techniques may be sensitive, but are also labour intensive. Electron microscopy is valuable but requires skilled staff and high cost equipment. Immune electron microscopy improves the sensitivity of the use of the electron microscope but is not suitable for routine use. Serological methods involving precipitation reactions can be modified to cope with large numbers of samples but have serious disadvantages.

ELISA is the only test in common use which is ideal for virus indexing, however it still presents some problems. Care must be taken in the design of the sampling and test procedure to ensure full reliability. Safeguards must be built in to warn of problems due to low virus concentrations. The limits of the antisera in use in detection of strains, must be appreciated. For each new virus/host combination, some investigation may be necessary.

For the future, ELISA may be made more sensitive with the use of alternative methods, monoclonal antibodies and alternative enzyme/substrate combinations.

INTRODUCTION

Of all plant pathogens, viruses are the most effectively passed from parent to progeny when plant material is vegetatively propagated. Justifiably, therefore, viruses, or particularly the elimination or avoidance of infection by viruses, has been of major concern in systems for the production of plant material by vegetative means and schemes for certification or monitoring the health of such material usually include some concern for virus disease.

In such schemes the detection of viruses often depends on recognition of symptoms caused by virus infection in the growing plants. For many good reasons a growing season inspection is an essential part of these schemes, not least because it ensures that requirements such as

varietal purity, trueness to type and freedom from diseases other than those due to viruses are met. In the course of crop inspection, whether done for certification purposes or as a grower precaution, virus infected material may be seen and rogued out.

However, whilst such inspections remain essential, they are not always reliable in detecting virus infection. Symptoms due to viruses may be confused with those caused by nutrient deficiency, environmental stress, or by senescence. Where propagation of crops cannot be in insect-proofed glasshouses, gauze houses or growth rooms, virus spread by insect or other natural vector may not be detected visually. Virus infection in plants may cause a variety of symptoms some of which may be easily recognised, but others may be indistinct, mild or no symptom may be produced at all. Thus alternative methods for detection of viruses are essential.

Such methods for detection of viruses in plant material during propagation must fulfil a number of essential criteria if they are to be of any use. Perhaps the most important quality required of tests for virus 'indexing' is that they should be highly sensitive, capable of detecting virus at low concentrations in plant tissue. Whilst sensitivity is vital, specificity need not be a quality of the test, provided its range of detection is known, within limits the flexibility to detect a range of viruses and strains of virus is an advantage. Techniques which demand skilled technicians are not ideal, and a high labour input is also undesirable. Tests must be practicable, reproducible and not too lengthy. Vegetative propagation methods by their nature generate large quantities of material, a significant proportion of which must be tested. The facility to test many samples quickly is therefore important.

THE RANGE OF TESTS AVAILABLE

The range of techniques for virus testing which fulfil the requirements defined above is limited. Tests involving transmission of viruses, whether mechanically, using the natural vector or by grafting may be sensitive if properly carried out but they are lengthy, labour intensive and unsuitable for testing large numbers of samples. Detection of viruses which occur naturally in high concentration in their host can be readily achieved by visualisation in the electron microscope using simple preparation methods, but whilst these methods can be quick they are not appropriate for large sample numbers and are not very sensitive. Improvements in the sensitivity and specificity of virus detection by electron microscopy can be achieved by combining the use of serological techniques and electron microscopy in the immune electron microscope (IEM) procedure. However, such improvements are at the expense of the rapidity of the test, and a higher level of skill and greater capital outlay is required. Serological techniques themselves, until comparatively recently, have had limited value in routine virus testing. The more traditional techniques involving the formation of visible precipitates when antibody and antigen (virus) mix in optimum conditions, have been modified for routine use. In the

microprecipitin test (Van Slogteren 1955) the mixing of antiserum dilutions and antigen-containing sap is reduced to the scale of small drops on a microscope slide, the precipitation reaction being viewed by light microscopy. Whilst offering some advantages, this sort of test, and others which can be similarly adapted to deal with large numbers of samples such as the latex test (Abu Salih et al.), have disadvantages which make them less convenient. In these methods the indication of virus presence requires a precipitation reaction which may not occur if either antibody or antigen is present in excess. Thus some degree of experience or preliminary investigation is needed in using such methods.

Of the methods presently available which are suitable for large scale virus testing, only the enzyme-linked immunosorbent assay (ELISA) fulfils the criteria defined above. This technique, described by Clark and Adams in 1977 has been extensively reviewed. It has the advantages of being sensitive and quick, and is capable of testing large numbers of samples. It is economical in use of antisera and may be semi-automated (Hill 1984a).

THE ELISA TECHNIQUE FOR VIRUS INDEXING PLANTS

ELISA is already in widespread use for the monitoring of the health of vegetatively propagated plant material and seeds. The method most widely used is that first described by Clark and Adams and commonly known as the double antibody sandwich ELISA. However modifications to this method which confer particular advantages have been described and reviewed (Hill 1984a). Detailed description of the techniques will not be provided here but can be found in appropriate texts (Hill 1984b). It is the interpretation of the results of ELISA tests and their use in procedures which ensure reliability which is most important when indexing plant material. These can be illustrated by reference to examples of the use of the ELISA test in practical situations.

ELISA is probably most frequently used in health monitoring in UK in the testing of top fruit during propagation for a range of viruses. Work by Torrance and Dolby (1984) defined the optimum conditions for sampling such material for testing in large scale surveys. Best results for detection of prune dwarf virus (PDV), prunus necrotic ringspot virus (NRSV) and apple mosaic virus (ApMV) were obtained when young leaves were tested. However the extent to which the virus could be detected within single infected leaves amongst a number of healthy leaves varied according to the virus being detected, the host being sampled and the time of sampling. Generally the more mature the leaves being tested, the less reliable the detection. Two important principles are illustrated by this experience.

Firstly, for a test to be effective in indexing vegetatively propagated material it must be sensitive enough to detect single infected samples amongst many healthy ones, to allow bulk testing to

be reliable and the capability of the test to be maximised. Thus the reaction in the ELISA test of the virus in single leaves must be good enough to permit dilution by at least ten times and preferably more, and yet still give positive results. Only ELISA and IEM techniques currently achieve this degree of sensitivity.

Secondly, the test must incorporate controls which can be used in interpretation of the results. These must consist not only of uninfected samples which provide a baseline for comparison, but also of a range of dilutions of infected material by which the sensitivity of the test at each dilution of infected samples in healthy can be judged. Material which is known to be infected and which can be stored freeze-dried provides a valuable standard between one test batch and another.

A disadvantage of the DAS-ELISA method is its specificity. Koenig (1978) has shown how the basic method described by Clark and Adams is very specific and may fail to detect closely related viruses or strains of virus. This is a disadvantage in routine screening, where virus identification may not be needed and so far as possible, ensuring virus absence is paramount. Thus the ELISA modifications such as indirect ELISA, described by many authors (Koenig 1981) may be better. In this respect, the use of monoclonal antibodies may present problems. These have the advantage of causing reactions in ELISA tests only to viruses, there is little or no background reaction as when polyclonal antisera are used. However monoclonal antibodies can be very specific, consequently care must be taken to avoid this when these are to be used for routine indexing. ELISA in all its forms is a test which detects only the target virus and not other unrelated viruses which may be present. Thus a degree of foreknowledge of the viruses likely to be of concern is necessary.

Whilst the ELISA technique can be used to test many samples, and many of the component stages in the ELISA test can be automated, the preparation of samples must still be done individually. Thus, so far as possible, if the advantages of the ELISA test are to be used, some mechanisation of sampling must be achieved. For leaf material, sap extraction using mechanical roller presser is efficient, dilution of the expressed sap in buffer may be achieved by dispensing buffer straight on to the rollers from a dispensing syringe. Sample extraction from material such as tuber flesh (potatoes, bulbs etc) is less easy. One device which achieves this and also dilutes the extract and dispenses it into the ELISA well, is the 'testbohrer' devised by Gugerli (1979) specifically for the sampling of potato tubers for virus test, and manufactured in West Germany. This consists of a modified dentist's drill which as it penetrates the tuber, displaces sap which is sucked into a tube held close by the drill tip. The sap extract is then dispensed into the ELISA well and the tube flushed out with buffer, so diluting the extract in the same process. Dried seeds present another difficult sap extraction problem. Even after night soaking these are still quite resilient. Compressing seeds within a strong polythene bag provided the best method for sap extraction but this was far from ideal.

CONCLUSION

Of all the virus tests presently available, ELISA is best for routine testing of large quantities of plant samples for virus. However, it must be undertaken with a full knowledge of its limitations. Although the procedure is comparatively simple, it is essential to have a knowledge of the viruses being sought, and their distribution and concentration in the host. The test must be arranged in such a way as to demonstrate its own reliability at each stage, all the appropriate standards and controls must be used. Despite the equipment which can be purchased to automate ELISA testing, sample preparation remains a time-consuming component of the test and mechanisation of this is important. The viruses affecting many hosts are not fully characterised and antisera not yet available so that ELISA cannot be used. For these, the traditional methods may still be the best available. For the future, the use of modifications of ELISA and monoclonal antibodies in ELISA should improve sensitivity. Pressure from industry will ensure that the antisera needed are produced.

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DETECTION OF VIRUSES AND VIROIDS IN PLANTS

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ABSTRACT

The detection of viruses in plants is becoming of increasing importance. The current biological and serological methods for plant virus detection have several limitations. The newly developed dot blot hybridization techniques are described and their potential for the future discussed.

INTRODUCTION

Viruses and viroids can cause considerable losses in both annual and perennial crops (see Hull, 1984). Epidemics can build up rapidly in monocultures of annual crops. In perennials and vegetatively propagated plants the accretion of several viruses can lead to the debilitation of the crops. The most common control methods are chemical ones against vectors for viruses of annual crops and roguing or eradication for viruses of vegetatively propagated and perennial crops. It is therefore important to have accurate and rapid means for the detection and identification of viruses.

Current methods

The two types of technique most commonly used for virus identification involve either biological or serological methods. The biological methods include sap inoculating mechanically transmitted viruses to a range of indicator plants, transferring insect vectors from infected plants to indicator plants and grafting also onto indicator species. All these techniques require a considerable amount of glasshouse or screened house space to maintain stocks of healthy plants and plants under test; the latter have to be well spaced to avoid cross-contamination. There are considerable phytosanitary risks in handling large numbers of infected plants. The raising and maintaining of the plants is very labour intensive and it can take a long time (up to months or even years) for results to be obtained.

Serological methods are based on the fact that, in most plant viruses, the nucleic acid is surrounded by a coat protein. This acts as an antigen when virus preparations are injected into animals. The elicited antibodies can be obtained and these react reasonably specifically with the antigen. Colour tests, such as the widely used enzyme-linked immunosorbent assay (ELISA) can be performed relatively rapidly and many samples can be routinely screened. A major drawback of serological methods is that they cannot be used to detect viroids or viral nucleic acids which do not become encapsidated in coat protein. Viroids, causing diseases such as chrysanthemum stunt or potato spindle tuber, are small, naked nucleic acids. Some viruses, e.g. tobacco rattle virus in potatoes and bulb crops, lose the ability to produce coat proteins.

Dot blotting

Recent developments in molecular biology have led to a range of techniques which can overcome these problems (for reviews see Hull, 1984; Hull, 1985). The basis of these techniques is nucleic acid hybridization which takes place between complementary strands of nucleic acid. Thus a plant virus nucleic acid, be it DNA or RNA, will hybridize under the appropriate conditions with a probe nucleic acid made up of sequences complementary to it. In practice the viral nucleic acid is immobilized onto a solid medium

as a spot or dot (hence the terms for the method - spot or dot blot hybridization). It is not necessary to purify the virus; just a spot of sap from an infected plant is needed. The probe is made by using enzymes to copy the viral nucleic acid and is often 'immortalized' by cloning it into a bacterial plasmid. The probe is labelled so that it can be detected after hybridization. Initially this was by radioactivity but recently non-radioactive colourimetric methods have been developed. The probe is hybridized to the viral nucleic acid and is then detected by its label.

The dot blot technique is as sensitive as, if not more sensitive than, the serological methods. It has been used to detect viruses in insect vectors (Boulton and Markham, 1985) which opens up the possibility of being able to reliably predict the spread of viruses. As well as overcoming the problem with non-encapsidated viruses and viroids it is potentially a much more powerful technique than serology. The genetic information used in eliciting an antiserum represents only a small fraction of the total information carried by the viral nucleic acid. The hybridization techniques use all, or any part required, of the viral nuceic acid. There are suggestions that, in the future, probes can be made for different needs, ones which are virus-specific for broad-range detection (e.g. quarantine or general screening) and ones which are strain-specific for studying, for example, epidemiology. We are only just beginning to realize the potential of these techniques. There are sure to be some major developments over the next few years.

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Poster Papers

THE PRODUCTION OF VIRUS-TESTED NARCISSUS IN SCOTLAND

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ABSTRACT

The scheme operating in Scotland aims to evaluate the benefits of freedom from viruses and to assess the practicability of maintaining the health of virus-tested narcissus stocks during field propagation. Virus-free mother plants are first obtained by selection, meristem-tip culture or twin-scale therapy. These are multiplied in vector-proof houses by two cycles of twin-scaling producing c. 20 000 three-year old bulbs annually for field propagation, which began in 1979 under conditions specified by the Foundation Stock Certification Scheme of the Department of Agriculture and Fisheries for Scotland. Visual inspection and virus-indexing of these stocks after the full six-year period of Foundation Stock propagation indicate that no detectable virus infection has yet occurred. An annual out-put of about 15-20 tonnes of Foundation Stock to members of the Scottish Nuclear Stocks Association (Flower Bulbs) Ltd. for commercial production began in 1985. Agronomic evaluation trials indicate that virus-tested stocks give higher yields and a greater proportion of the premium, larger sized bulbs than commercial stocks.

INTRODUCTION

Since 1969 Scottish farmers have used the Narcissus Certification Scheme organised by the Department of Agriculture and Fisheries for Scotland (DAFS) to improve and maintain the health of their stocks. This Scheme, based on visual inspection and rogueing, controls some viruses and provides the basis for the health standard required for bulb exports. As such, it continues to be well supported by growers.

Research showed, however, that several viruses infect narcissus either without causing obvious symptoms, or alternatively not causing symptoms in all cultivars or at all times. Thus a virus-tested stocks scheme was required to produce a further improvement in health. In 1972 such a programme was initiated for the Scottish industry by joint agreement between, and participation of, the Scottish Crop Research Institute, DAFS, the Scottish Colleges of Agriculture and growers represented by the Scottish Nuclear Stocks Association (Flower Bulbs) Ltd. (SNSA(F.B.)Ltd.).

The scheme was designed both as a commercial and experimental operation and its main objectives were:

1) to obtain virus-free mother plants of the most important narcissus cultivars

2) to establish a propagation scheme providing a continuous supply of virus-tested stocks to the industry

3) to design and assess measures to prevent the viruses re-infecting stocks during propagation

4) to characterise the viruses and virus diseases affecting narcissus and to devise reliable methods of virus detection suitable for testing the health of stocks

5) to evaluate the agronomic benefits of freedom from viruses

VIRUS-FREE MOTHER PLANTS

Initially the SNSA(F.B.)Ltd. chose 12 major cultivars for entry into the Scheme. Virus-free mother bulbs representing these cultivars were obtained by three methods, virus-indexing of commercial stocks, twin-scale therapy and meristem-tip culture (Mowat 1980). The first method provided the plants available for propagation soonest but succeeded with only six cultivars: viz. Barrett Browning, Carlton, Corinthian, Red Goblet, Sempre Avanti and Verger. The other 6 cultivars were apparently totally virus infected, all but one (cv Yellow Cheerfulness) with a potyvirus which was code named BA3-8 virus and is apparently associated with late season yellows disease (Mowat & Duncan 1985). Virus-free mother bulbs of these six cultivars, Dutch Master, Fortune, Golden Harvest, King Alfred, Rembrandt and Yellow Cheerfulness were obtained by meristem-tip culture and/or twin-scale therapy. Some mother plants of the cultivars in the first group were also obtained by these means. Eventually each cultivar was represented by several clones which are kept separate during propagation both as a measure in the management of virus-indexing and to provide the opportunity for clonal comparisons.

PROPAGATION SCHEME

The scheme comprises four stages, designated the first, second and third stages of propagation and commercial production. Propagation in the first and second stages is by twin-scaling (Mowat & Chambers 1975, Mowat 1980) based on the procedures of Alkema (1970, 1971, 1975 and pers. comms) and Tompsett (1972). The target weight of bulbs in a clone at the end of the first stage of propagation is 5-7 kg. The minimum period for stage 1 propagation is 4 years. Stage 2 propagation began in 1976. Each year clones with a total bulb weight of c. 25 kg of cultivars chosen by SNSA (F.B.)Ltd. are multiplied by twin-scaling and the plants grown for 3 years for the bulbs to attain a size suitable for planting in the field (stage 3). Output from stage 2 propagation is c. 20 000 bulbs of total weight c. 400 kg. Thus from each 1.25 g of bulb twin-scaled, one bulb averaging 20 g is obtained in 3 years. The final stage of propagation is by three biennial cycles of natural multiplication in the field producing about 15-20 tonnes of bulbs (Foundation Stock) per annum to be sold to members of SNSA(F.B.) Ltd. for commercial production. Stage 3 propagation began in 1979 and the annual distribution of Foundation Stocks began in 1985.

PROTECTION FROM VIRUS INFECTION

Of the 12 commonly occurring viruses likely to spread in commercial stocks of narcissus in the UK up to nine may have the potential to infect virus-tested narcissus stocks in Scotland. Four are transmitted by aphids (narcissus latent, BA3-8 (associated with late season yellows), narcissus yellow stripe and narcissus white streak) and three by nematodes (tomato

black ring, raspberry ringspot and tobacco rattle). The mode of spread of two, narcissus mosaic and narcissus tip necrosis, is unknown.

Throughout rapid multiplication in the first and second stages of propagation, plants are kept in houses designed to keep out nematode and aphid vectors. Nematodes are excluded by using either a soil-less compost or soil fumigated with methyl bromide placed in beds lined with polythene sheet. Protection against aerial vectors is provided by a gauze cover of a mesh size which will exclude even wingless aphids (e.g. Tygan, T151/000/00, Fothergill & Harvey Ltd, UK). On release for field propagation, samples from 1 000 of the 20 000 plants are routinely indexed by enzyme-linked immunosorbent assay (ELISA) for narcissus mosaic and narcissus tip necrosis viruses. Neither virus has been detected in the seven issues made so far.

In the third stage of propagation several precautions are taken to prevent infection by nematode- and aphid-borne viruses. To avoid infection by arabis mosaic and strawberry latent ringspot viruses, stocks are grown in that region of Scotland (north of the River Tay) outside the northern limit of distribution of their nematode vector, Xiphinema diversicaudatum. To prevent infection by the other nematodeborne viruses, every year several sites, chosen by the SNSA(F.B.)Ltd., are examined for vector nematodes (Longidorus elongatus and trichodorids) and soil samples are tested for tomato black ring, raspberry ringspot and tobacco rattle viruses by growing bait plants in them. Sites with virus-infective soil are rejected; from the remainder, the site with the lowest nematode vector count is selected, with preference being given to sites where there are few or no trichodorids. In addition, the soil is fumigated with dichloropropene (Telone II) applied by a Rumptstad Combiject at 225 litres of product per ha. To control infection by aphid-borne viruses, all of which are retained only briefly by the aphids (non-persistent type) and do not infect native wild plants in Scotland, sites must not have grown narcissus previously and in addition must be at least 500 m distant from non-virus-tested stocks of narcissus. These requirements are specified by DAFS for the Certification of Foundation Stock. The results from annual visual inspection and virus-indexing of the first issue of virus-tested bulbs (clones of cvs Carlton and Sempre Avanti) have provided no evidence of virus spread into the stocks during the full 6-year period of Foundation Stock propagation (see Table). Sufficient tests were done in 1985 to indicate that the incidence of infection, if any had occurred, was less than 0.5% (probability level of 99%).

VIRUS DETECTION

The successful development and operation of a scheme for producing virus-tested stocks depends on the reliable detection of infected plants by methods suitable for application on a large scale. Because symptom diagnosis is of limited use in narcissus other means of detection are required.

Of the nine viruses which may have the potential to spread in Scotland, antisera are available to seven. Assessment of ELISA as a sensitive serological test suitable for screening virus-tested stocks for these seven viruses showed that the method was satisfactory for six; narcissus latent, BA3-8 (associated with late season yellows), tomato black ring, raspberry ringspot, narcissus mosaic and narcissus tip necrosis (Mowat 1986). However, ELISA seems unsuitable for detecting tobacco rattle virus because of the antigenic variability of isolates (Harrison et al. 1983, Mowat 1986).

TABLE

Virus-indexing of virus-tested stocks of cvs Carlton and Sempre Avanti during field propagation at Foundation Stock Certification grade.

Growing season	Virus[+]						
	NMV	NTNV	TBRV	RRV	TRV	NLV	BA3-8
1980	-	-	-	-	-	-	-
1981	0/480[E*]	0/480[E]	0/100[I]	0/100[I]	0/100[I]	-	-
1982	0/500[E]	0/500[E]	0/200[I]	0/200[I]	0/200[I]	-	-
1983	0/500[E]	0/500[E]	0/100[I]	0/100[I]	0/100[I]	-	-
1984	0/500[E]	0/500[E]	-	-	-	-	-
1985	0/920[E]	0/920[E]	0/920[E]	0/920[E]	-	0/920[E]	0/920[E]

[+] narcissus mosaic = NMV; narcissus tip necrosis = NTNV; tomato black ring = TBRV; raspberry ringspot = RRV; tobacco rattle = TRV; narcissus latent = NLV.

[*] Numerator is the number of plants infected; denominator is the total number of plants tested. Samples were taken at equal intervals throughout the planting.

E = indexed by ELISA; in 1985 500 samples tested in batches of 10 and 420 samples in batches of 2; in other years samples tested in batches of 25.

I = samples tested individually by inoculation of sap extracted in 0.07 or 0.03 M phosphate buffer, pH 7 (6 ml/g of leaf) to Chenopodium amaranticolor and C. quinoa. (Data from Mowat 1986).

Although this virus can be detected satisfactorily in narcissus by spot hybridization tests using complementary DNA (Robinson 1985) this method is not yet developed to a stage fully suitable for routine screening. Meantime detection of tobacco rattle depends on the production of symptoms in narcissus or after inoculation of narcissus sap to indicator plants. Detection of narcissus yellow stripe virus (a potyvirus) and of the narcissus white streak agent (probably a virus) also depends on symptom production in narcissus; neither specific antisera nor indicator hosts are available for these two viruses/agents.

AGRONOMIC ASSESSMENT

First results from a continuing series of field trials have shown that bulbs of virus-tested stocks of cv Carlton grown for two years in stage 3 propagation and then grown for 2 years alongside two commercial stocks gave a greater increase in bulb weight (c. 20%) and produced a greater proportion (c. 5-fold) of bulbs >15 cm in circumference (Sutton et al. 1986, Sutton, unpublished data). Similar results were also obtained with cv Sempre Avanti in another trial (Sutton et al. 1986, Sutton, unpublished data). However, when bulbs from the same clone of cv Carlton were produced at two locations in stage 2 propagation and then grown for 2 years

at the same site during stage 3 propagation (that is 5 years after twin-scale propagation), bulbs from the two sources performed differently. Thus the differences observed between virus-tested and commercial stocks of cvs Carlton and Sempre Avanti may not be solely attributable to the effects of a high incidence of narcissus mosaic and narcissus tip necrosis viruses in commercial stocks of these cultivars (Mowat 1984, 1985).

DISCUSSION AND CONCLUSIONS

With the start in 1985 of the annual sale by SNSA(F.B.)Ltd. of c. 15-20 tonnes of Foundation Stock to members of the association, the propagation scheme has become fully functional. The Foundation Stocks will now be grown under commercial conditions and will be eligible for certification at Elite grade which again requires that they are grown under conditions designed to minimise virus infection (see P.A. Rankin, Narcissus Certification in Scotland, this publication). Although in recent years trial batches of VT narcissus have been propagated by chipping (Flint 1982) and by tissue culture methods (Harper & Fordyce, unpublished data), routine propagation has been entirely by twin-scaling. Throughout the use of this method (since 1972 for stage 1, and 1976 for stage 2) very few plants with flower abnormalities have been noted. Almost all defects were splits in the corona and these as well as the one example of pigment aberration reverted to normality. Twin-scale propagation therefore seems not to produce variant forms. In addition, results from evaluation trials have provided no evidence that virus-tested plants of cvs Carlton and Sempre Avanti are more variable in growth and development than plants of commercial stocks (Sutton et al. 1986).

The results of virus-indexing Foundation Stocks in the sixth year of propagation at this grade (see Table) suggest that the measures applied to prevent infection by aphid-borne and nematode-borne viruses are very effective. The failure to detect narcissus mosaic and narcissus tip necrosis viruses in stage 2 and field propagation is particularly encouraging as the mode of spread of these two viruses is unknown and yet they occur commonly in commercial stocks of some cultivars such as Carlton (maximum incidence 40% for narcissus tip necrosis virus and 80% for narcissus mosaic virus) and Sempre Avanti (maximum incidence 90% for narcissus tip necrosis virus and 73% for narcissus mosaic virus) (Mowat 1984, 1985). It has been postulated that these viruses may be spread as a result of defoliation by flailing and, until results from trials to test this hypothesis are available, flailing of virus-tested stocks is prohibited. The aphid-transmitted BA3-8 virus is probably by far the most prevalent virus in commercial narcissus stocks. Preliminary data (Mowat, unpublished) indicate that although cultivars such as Dutch Master, Golden Harvest, King Alfred and Rembrandt are totally infected, BA3-8 virus may not infect other cultivars such as Carlton and Sempre Avanti. Thus the conditions presently applied to prevent infection by aphid-borne viruses may be more rigorously tested when stocks of cultivars susceptible to this virus are available.

Agronomic evaluation has, as expected, proved to be a complex task because, unlike some other vegetatively propagated crops such as potatoes, narcissus plants that are grown under different environmental conditions cannot be brought to the same physiological condition by a brief conditioning treatment. An additional complexity is the large number of viruses and cultivars, any pair of which may interact differently from other pairs.

However, the indications so far are that a main effect of freedom from infection may be to increase the proportion of bulbs in the premium, larger size grades. This seems to be the effect not only of freedom from narcissus mosaic and narcissus tip necrosis viruses in cvs Carlton and Sempre Avanti but also of freedom from narcissus yellow stripe virus in cvs Carlton and Helios (Thompson et al. 1982). However, because of the large effort needed to obtain them, such data from experiments on the effects of viruses are likely to become available for only a few combinations of narcissus cultivars and viruses. A further assessment of the benefits and commercial viability of virus-tested stocks will be possible when this material has been grown for a few years under commercial conditions. With the distribution of Foundation Stocks, this economic assessment has now begun.

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THE IDENTIFICATION OF SOURCES OF FIELD IMMUNITY FROM POPLAR MOSAIC VIRUS

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ABSTRACT

Three methods of detecting poplar mosaic virus (PMV) were compared using a range of poplar genotypes. Visual inspection of foliar symptoms and infectivity assays for PMV in leaf extracts inoculated to Nicotiana megalosiphon were less reliable indicators of infection than were ELISA tests for viral antigens. Infection with conspicuous leaf symptoms was common in Aigeiros species or hybrids, particularly in Populus deltoides. Bioassays rarely detected PMV either in the mainly symptomless Tacamahaca clones or when P. nigra was the Aigeiros parent. The leaves of Tacamahaca species manually inoculated with PMV-containing N. megalosiphon sap were not infected, even locally, and results of the challenge by chip bud inoculation with a source of PMV in P. nigra are awaited.

INTRODUCTION

Poplar mosaic virus (PMV), a carlavirus, is ubiquitous in some commercial poplar clones and has been associated with 30-40% diminishment of growth rate. The prevalance of the virus is partly attributable to vegetative propagation of infected stock but, since the virus is not transmitted via seed, the occurence of infection in breeders selections that were initially virus free seedlings, implies that spread occurs naturally (see Cooper & Edwards 1981). The leaf symptoms caused by PMV in poplar are distinctive yellow star shaped (asteroid) spots. However, the occurence of these lesions is influenced by the cultivar, the season, the climate and the virus pathotype. Consequently symptoms are often transient and sometimes completely absent from infected trees (Cooper & Edwards 1981).

Detection of virus particles is possible if leaf squashes are examined with an electron microscope using phosphotungstic acid (pH 7.0) or uranyl acetate (pH 7.3) as negative stains but the occurence of flexuous tubular particles with a modal length in the range 626-735nm is not unambiguous because a potyvirus has also been identified in poplar (Martin **et al.** 1982). A third method for PMV detection is a bioassay (infectivity) test. PMV is routinely transmissible by manually inoculating foliage of Nicotiana megalosiphon Heurk. and Meull. with poplar sap. N. megalosiphon plants show veinal necrosis in leaves formed after inoculation. Berg (1964) used these three methods in parallel when surveying for PMV and recognized that the virus was more common in clones/species assigned to the section Aigeiros than in representatives of the section Leuce, Leucoides and

Tacamahaca. Bioassays and electron microscopy, although sensitive methods, are time consuming and visual assessments of PMV infection are not completely reliable. Consequently, the value of ELISA detection has been assessed using poplar clones grown as trees (Cooper & Edwards 1981) and in stool beds (van de Meer **et al.** 1980). These studies showed that naturally infected poplars contain populations of PMV isolates indistinguishable using ELISA but differing in their pathogenicity for poplar or invasivness in Chenopodium quinoa Willd. Furthermore, the unequal distribution of PMV in foliage along branches presented sampling problems which could be partially overcome by pooling samples and by selecting the most reliable sources of material for testing.

Here we report the results of a survey in which our main objective was to identify sources of field immunity from PMV. Concomitantly we compared the reliability of visual, ELISA and infectivity tests as criteria for inferring the presence of PMV in a large range of traditional commercial clones and promising advanced selections under trial in Poland and England.

MATERIALS AND METHODS

Poplars

The trees were species and hybrids of the sections Aigeiros, Tachamahaca, Leuce and Leucoides growing in stool beds in England and Poland. Long established commercial clones naturally infected with PMV had been liberally interplanted among the advanced selections for at least five years.

Virus tests

Foliage of each clone was examined for asteroid spots in summer and autumn. Winter buds and/or leaves were tested by ELISA and bioassay as described by Cooper & Edwards (1981).

Challenge inoculation of poplars

Carborundum dusted leaves of rooted cuttings in which PMV had not been detected were manually inoculated in July with a PMV isolate (ATCC PV275) propagated in N. melagosiphon. Trees were kept in an insect proofed glasshouse before and after inoculation and, in September, inoculated leaves were tested for infectious virus.

RESULTS

Unambiguous evidence for PMV infection was found in 24% (84/344) of the clones in England and 56% (143/254) of the clones in Poland. In general, ELISA data indicated a higher proportion of infected clones (48%, 124/287) than either visual assessment of foliar symptoms (34%, 200/595) or bioassay (27%, 19/71). However, in several instances, ELISA tests on buds did not detect the presence of PMV in poplars having prominent foliar symptoms and some poplar clones lacking foliage symptoms were ELISA

positive (see Table 1).

Bioassays on freshly collected leaves were in close agreement with ELISA tests done on leaves/buds from the same clone (5/33 positive by bioassay; 6/33 positive by ELISA). However, results of bioassays on leaves which had been in transit from Poland to UK for several days were in poor agreement with ELISA data (14/38 positive by bioassay; 29/38 positive by ELISA).

The prevalence of virus varied among taxa (Table 2). Both visual assessment and ELISA data indicated that infection was more common in the species and hybrids assigned to the Aigeiros section than in those of the section Tacamahaca. No evidence for infection was found in Leuce and Leucoides. Within the section Aigeiros, symptoms were observed in a larger proportion of P. deltoides clones (15/25) than in clones of P. nigra (9/57). However, whereas ELISA tests did not detect further infection in P.deltoides (12 clones tested), ten of 39 additional infected clones of P.nigra were ELISA positive.

TABLE 1

ELISA tests on buds from poplars lacking foliage symptoms

Clone	Absorbance values					
	Tip buds combined from 5 shoots	Lateral buds from each of 5 shoots				
		1	2	3	4	5
P.nigra PW-2	0.95	1.50	1.50	1.50	1.50	1.28
P.nigra Janinow	0.08	0.28	0.04	0.21	1.05	0.42
P.nigra Jalta	0.00	0.68	0.13	0.06	0.06	0.06
P.deltoides x P.nigra	0.41	1.38	0.02	0.47	0.34	0.24
P.trichocarpa W-29	0.03	0.03	0.07	0.05	0.05	0.04

TABLE 2

Prevalence of PMV in different Sections

Section	No of clones infected/No of clones tested	%
Aigeiros x Aigeiros	162/279	58
Aigeiros	34/95	60
Tacamahaca x Tacamahaca	5/50	10
Tacamahaca	10/87	11
Leuce, Leuce x Leuce, Leucoides	0/25	0

Infection in the intersectional hybrids (Aigeiros x Tacamahaca and Tacamahaca x Aigeiros) were more common in clones having P.deltoides as a parent than in those having P.nigra (Table 3).

TABLE 3

PMV in intersectional hybrids with P.deltoides or P.nigra as parents.

Hybrid	No. of clones infected/No. of clones tested
P.deltoides angulata cordata P. x berolinensis	3/3
P.deltoides x P.trichocarpa	4/6
P.nigra 'Italica' P. x berolinensis	0/2
P.nigra x P.lauriflolia	0/7
P.nigra x P.trichocarpa	2/4
P.maximowiczii x P.nigra	1/11
P.maximowiczii x P.nigra	2/7

Leaves of PMV-free clones (Tacamahaca hybrids and hybrids between P. nigra and Tacamahaca species) mechanically inoculated with PMV-infected N. megalosiphon sap did not become infected as judged by bioassay tests.

DISCUSSION

Laboratory tests are more expensive and demanding of skilled labour than field observations. Furthermore, under appropriate weather conditions and for certain combinations of poplar genotypes and virus pathotypes, recognizable symptoms are produced and are an adequate indicator of PMV infection. ELISA which is convenient and rapid, detected symptomless invasions that seemed common in P. nigra and in Tacamahaca species. It must be emphasized, however, that more than one test was in some instances needed to confirm an initial negative result perhaps because the concentrations of ELISA detectable antigen vary amongst buds and in leaves (van der Meer et al. 1980).

Our results, like those of Berg (1964) indicate that Aigeiros poplars are frequently infected with PMV. We tested a greater range of Tacamahaca species and hybrids than did Berg but, even in the Polish collection which was extensively tested by ELISA, noticed relatively few PMV-infected clones. A few species/cultivars in the section Tacamahaca are reported to be experimentally infectable with PMV (Berg 1964) but our initial attempts to mechanically infect other Tacamahaca cultivars (eg.P. maximowiczii) failed. A more critical assessment of the infectability of different genotypes following chip bud inoculation is currently underway.

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NURSERY TREES OF STONE-FRUITS FREE FROM BACTERIAL DISEASES

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ABSTRACT

New approaches are being developed to combat bacterial plant diseases, always notoriously difficult to control due to the dearth of effective bactericides. Biological control of crown gall (Agrobacterium tumefaciens) using the Australian strain 84 of A. radiobacter as antagonist has become widely used. It is shown to be effective in production of gall-free nursery trees of the cherry rootstock Colt. However, not all strains of the pathogen are sensitive to this antagonist.

Plum trees are most susceptible to bacterial canker (Pseudomonas syringae pv morsprunorum) when young. Most infections occur on the trunk and can cause cankers which girdle it so killing the tree. In an attempt to produce trees free from bud infestation with the pathogen, which provides the source of inoculum for infection, an orchard of micropropagated Victoria plums has been established. It is hoped that these will remain pathogen-free during the early years when susceptibility to canker is high.

INTRODUCTION

Two problems in nursery production of stone-fruit trees are the bacterial diseases crown gall (caused by Agrobacterium tumefaciens) and bacterial canker (caused by Pseudomonas syringae pv morsprunorum).

A. tumefaciens, a soil-inhabiting bacterium that attacks a wide range of hosts, enters the plant through wounds, usually at the crown or on the root. It induces the unregulated cell division in the host that leads to gall formation (Lelliott 1975).

Infection of stone-fruits occurs most readily in spring or early summer and gall tissue develops most rapidly when host activity is maximal. Nursery trees lifted in the dormant season may be visibly galled making the tree unsaleable. In addition, adjacent apparently healthy trees, or those from a site on which the disease is not yet evident, may have become infected late in the season and therefore be symptomless when lifted. Nevertheless, these may develop galls when host plant activity resumes in the spring (Garrett 1978).

Bacterial canker affects the aerial parts of the host plant. P. syringae pv morsprunorum, a widely distributed leaf surface epiphyte on orchard stone-fruit trees, provides the inoculum for autumnal leaf scar infection of cherry. This leads, in early spring, to necrosis of buds and spurs and later to death of even large branches (Crosse 1963). An autumn spray schedule of Bordeaux mixture gives effective control (Garrett 1980). A different situation pertains on plum. On this host, trunk wounds on young trees, up to 6-8 years old, may become infected in late autumn or winter. Resultant cankers can extend the length of the trunk and girdle it, so killing the young tree. As yet there is no satisfactory control of the disease on plum (Garrett 1980). The source

of this inoculum is the nursery trees that become infested from the population of the pathogen on the mother trees from which the bud- or graft-wood was derived. Thus the pathogen is introduced into a new orchard (Gunawardena 1983).

Bacterial diseases of plants are notoriously difficult to control and the dearth of satisfactory bactericides increases the problem. Some new approaches to disease control are presented that aim to ensure that nursery trees of stone-fruits are free from bacterial pathogens when planted in the orchard.

1. BIOLOGICAL CONTROL OF CROWN GALL

Control of crown gall, until a decade ago an intractable problem, became possible with the discovery that A. radiobacter strain 84 was antagonistic to the crown gall pathogen (New & Kerr 1972) through production of agrocin 84, a highly specific nucleotide bacteriocin (Kerr 1980). Strain 84, used commercially in Australia since 1973, is now used to protect plants against crown gall infection in many other countries, especially in stone-fruit and rose nurseries (Moore 1979). It is supplied either as impregnated peat or filter paper discs or as agar cultures.

Formerly almost all cherries in the U.K. were grown on F12/1 rootstocks. These were propagated in layer beds, in which crown gall could build up over 10-15 years and cause latent infections in a high proportion of the rootstocks derived therefrom. Now, the majority of cherries are grown on the more dwarfing Colt rootstocks, propagated from cuttings. This enables full advantage to be taken of the availability of strain 84.

METHOD AND RESULTS

Four hundred and twenty cuttings of the cherry rootstock Colt were dipped in water or in a suspension of A. radiobacter strain 84 (c. 10^7 cfu/ml) for 1 minute before lining-out in the nursery. One hundred and forty of each treatment were grafted in the following year with the sweet cherry cultivars Napoleon or Roundel. When these maiden trees were lifted one year later for transplanting to an orchard their root systems were examined for the presence of crown gall. Treatment with strain 84 gave almost complete protection (Table 1).

CONCLUSIONS

This method of control is simple (requiring no complicated equipment), inexpensive, selective and above all, reliable provided due cognisance is taken that live organisms will not survive suspension in chlorinated water, exposure to extreme temperatures or to direct sunlight.

Not only are cuttings protected at the time of lining-out but colonisation of roots and rhizosphere by strain 84, its natural habitat, ensures continued protection as the root system develops. A further treatment with the antagonist prior to planting in the orchard protects for at least 2 years (Garrett et al. 1985).

Unfortunately the method is not applicable to all crown gall problems in fruit since some strains of A. tumefaciens, e.g. those attacking apple and cane fruits, are insensitive to agrocin 84. However, on those

nurseries where crown gall is caused by strains of the pathogen that are sensitive to agrocin 84 healthy, gall-free planting material can be obtained by this method of biological control.

TABLE 1

Effect of treatment with A. radiobacter strain 84 on incidence of crown gall on nursery trees of cherry

Cherry	Trees galled (%)	
	Control	Treated with 84
Colt rootstock	13.47	-
Colt worked with Roundel	20.29	1.41
Colt worked with Napoleon	36.23	0.0

2. BACTERIAL CANKER OF PLUM: DISEASE AVOIDANCE

Much of the bacterial canker in young plum orchards originates from bud infestation of nursery trees. The bacteria are difficult to eliminate from mother trees and nursery stock by conventional sprays because the only ones available - formulations of copper - are phytotoxic. Progress in micropropagation methods has therefore been exploited in an attempt to produce pathogen-free trees. It is hoped that these, when planted in reasonable isolation from other plums, will maintain their pathogen-free status during their years of greatest susceptibility to canker.

METHODS AND RESULTS

Sterile shoot tips of plum, cultivar Victoria and rootstock Pixy, were maintained on the modified Murashige and Skoog medium containing phloroglucinol and 6-benzylaminopurine (BA) of Jones & Hopgood. Roots were induced by transferring single shoots to a medium without BA but containing 3 mg/l IBA (Jones and Hopgood 1979). Propagules were transferred to the glasshouse after 6 weeks, and carefully potted into sterile compost. They were maintained under humid conditions and, after careful weaning, transferred to a cold greenhouse. After one year's growth the Pixy stock was budded with the Victoria scion and grown on for a further year in the greenhouse.

These micropropagated maiden trees were used to establish an orchard at East Malling Research Station. Leaf samples of the trees, some in their first and some now in their second growing season, were taken at intervals throughout the summer (1985) to monitor their epiphytic population. So far the canker pathogen has not been detected on any of these trees. This trial is continuing to determine for how long the trees remain pathogen and disease free.

CONCLUSIONS

This approach to control of plum bacterial canker appears promising. On another plot of similar age on a distant part of the Research Station, established from trees propagated by traditional methods, 2 plum trees have already died from bacterial canker and the pathogen has been isolated from the leaf surfaces of others.

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SEED TREATMENT CONTROL OF PHOMA AND ALTERNARIA INFECTIONS OF BRASSICA SEED

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BACKGROUND AND OBJECTIVES

In recent years, canker (Phoma lingam - pycnidial state of Leptosphaeria maculans) (Humpherson-Jones 1983) and dark leaf spot (particularly Alternaria brassicae) have become prevalent in horticultural brassicas causing crop losses by spoilage of produce and occasionally plant death. This increase in incidence appears to be related to the direct transfer of these pathogens to horticultural crops from nearby infected oil-seed rape crops.

These fungi and additionally A. brassicicola, are seed-borne and are present in oil-seed rape and horticultural brassica seeds. The effectiveness of seed treatment with thiabendazole to control Phoma infection (Maude et al. 1973) and with iprodione to eliminate Alternaria from seeds (Maude & Humpherson-Jones 1981) has been reported. The present research describes the selection of fungicides active against all of these pathogens and their performance as seed treatments.

MATERIALS AND METHODS

Routine in vitro screening tests were made using a fungicide concentration range of 0.0156 - 2500 ug/ml in Coons Agar. The agar was autoclaved (15 p.s.i. for 15 min) in 80% of the water and the remaining water containing fungicide added as the agar cooled to give the concentration range cited. The fungi were added to the test media as 6 mm disks. Linear growth on four plates per concentration was measured when cultures on untreated agar covered 50% of the agar surface.

Fungicides were applied to naturally-infected brassica seeds. Their effects on infection were recorded on agar, on germination on moist cellulose pads on a Copenhagen tank; and on infection and emergence in unsterile soil in Dutch light frames. In the laboratory the agar, cellulose pads bearing 200 - 400 seeds were examined after 7 and 14 days. Soil tests were made over a period of 3 months with 1000 - 1200 seedlings examined and tested for infection on 3 sample dates at 28-day intervals. Pathogens were identified following direct isolation onto Prune Lactose Yeast agar.

RESULTS AND CONCLUSIONS

Fungicides which were completely inhibitory to fungal growth on agar at concentrations of 20 ug/ml or less usually had eradicant properties in seed treatment tests.

Benzimidazole-based and related fungicides were toxic to P. lingam but had little inhibitory effect, even at 1000 ug/ml, against Alternaria spp. in culture. As seed treatments they often increased its incidence in agar and cellulose pad tests presumably by destroying antagonistic seed surface organisms. Fungicides toxic to the three pathogens in culture included imazalil, fenpropimorph and iprodione all at 4 ug/ml and fenarimol and nuarimol at 20 ug/ml. These fungicides were applied to seeds as liquid or powder formulations at rates of 0.6125 to 5.0 g a.i./kg seed. Nuarimol and fenarimol at 1.25 g a.i./kg caused reduced germination and stunted

seedlings; imazalil produced a similar effect at 2.5 g a.i./kg. Reducing the concentrations of the three fungicides alleviated the adverse effects on germination but disease control was lost. Seed treatment with iprodione (1.25 to 2.5 g a.i./kg) and fenpropimorph (0.625-2.5 g a.i./kg) virtually eliminated all three pathogens in naturally-infected seed without affecting seed germination in laboratory tests. In seedling tests in unsterile soil (c. 3000 plants/treatment) the pathogens (P. lingam and A. brassicicola) were controlled by iprodione (2.5 g a.i./kg) and fenpropimorph (1.25-2.5 g a.i./kg). When green and red cabbage were grown from seeds treated with either fungicide healthy crops were produced. Neither fungicide, however, prevented pre-emergence damping-off infections due to Phycomycete fungi in soil but current tests of commercially formulated liquid seed treatment mixtures which, in addition to iprodione or fenpropimorph contain a broad spectrum fungicide and an insecticide (to reduce cabbage stem flea beetle) have proved successful.

The single fungicide approach has provided two chemicals, iprodione and fenpropimorph which can be formulated as seed treatments to eradicate Alternaria and Phoma infections from oil-seed rape and horticultural seed stocks.

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ENDOPHYTE INFECTED RYEGRASS SEED - THE STORY SO FAR

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ABSTRACT

Perennial ryegrass plants infected by an endophytic fungus were found in eight of 16 old grass swards surveyed in England and Wales. However, in plants grown from seed lots of perennial ryegrass cultivars currently available to farmers, endophyte infection was detected in only one of the 45 cultivars tested.

Infection of perennial ryegrass seedlings by the endophyte Acremonium loliae did not affect the level of attack by frit fly larvae. Seed treatment with the fungicide prochloraz gave good control of infection by A. loliae.

INTRODUCTION

Infection of grass plants by endophytic fungi has been known for many years but until recently it had been regarded as having little significance. But endophytes are now the subject of intensive research, particularly in New Zealand and the USA, for three reasons. Firstly, toxins produced by endophytes deter certain insect pests from feeding on infected plants (Prestidge et al. 1982; Latch et al. 1985). Secondly, other toxins produced by endophytes are now known to be the cause of the disorders 'ryegrass staggers' and 'fescue toxicity' in grazing animals (Fletcher & Harvey, 1981; Schmidt et al. 1982). These disorders have been a serious, although sporadic, problem in New Zealand and the USA for many years but the cause was unknown. Because the toxins which affect animals are different from those that deter pests, it may be possible to manipulate the endophyte to confer pest resistance without risk to the grazing animal. Also, grasses are grown widely in non-grazing situations and a perennial ryegrass cultivar high in endophyte is marketed in the USA for amenity purposes, where the pest resistance can be exploited with disregard to the staggers problem (Hurley et al. 1984).

A third reason is that in a pot experiment endophyte infection benefitted the herbage yield of perennial ryegrass (Latch et al. 1985). This finding has obvious implications for grass breeders.

Endophytes are seed-borne and seed treatment with fungicides can eradicate infection (Harvey, 1982). Infected seed appears to be the only means of dissemination in nature. Five species of endophyte have been identified in ryegrass and fescue (Latch et al. 1984). Perennial ryegrass is not indigenous to New Zealand and it is likely that the endophytes present there were introduced with seed from Britain. In New Zealand the main species involved

with staggers and pest resistance in ryegrass is Acremonium loliae, which is an anamorph of Epichloe typhina, the cause of 'choke' disease in grasses.

In Britain the only reports of endophyte presence in grasses were published many years ago (Sampson, 1935,1937,1939). In a small-scale survey of perennial ryegrass, five out of 42 plants collected were infected (Sampson, 1939). The endophytes were not identified, but the description of one of them apparently matches that of Acremonium loliae (Latch et al. 1984).

Staggers-like symptoms in grazing animals occur occasionally in Britain, especially in long-established sheep grazing areas, and in some cases there is circumstantial evidence that endophytes are responsible (P.G. Mantle, personal communication).

This paper gives the preliminary results of investigations into endophyte presence in old grassland in Britain and in commercial seed lots. Also endophyte was assessed for potential control of frit fly larvae (e.g. Oscinella frit) which are major pests in Britain.

METHODS

Experiment 1, Survey of old grassland for endophyte

Eight perennial ryegrass plants were collected from each of 16 sites in England and Wales during September 1984 (Fig. 1). The sites were being used for an experiment concerned with the improvement of old pasture and most of the swards were at least 20 years old (Hopkins et al. 1985). The plants were grown in a heated (c. 20°C) glasshouse and sampled for endophyte infection in April, 1985. To locate the endophyte, one tiller from each plant was removed and the outer leaf sheath, which usually was senescent, was removed and discarded. The next leaf sheath was removed and placed under a low power microscope with the inner surface facing upwards. A strip of epidermis was peeled off the sheath, placed on a microscope slide, and mounted in cotton blue/lactophenol stain. When the stain had permeated through the epidermal strip, the strip was examined under a high-power microscope for endophyte mycelium.

Experiment 2. Survey of commercial seed lots for endophyte

(i) Seed sown in pots in glasshouse

In February 1985, 25 seed lots from a total of 20 cultivars were obtained from seed suppliers. All but two cultivars were available on the British market. The two exceptions, Ellett, from New Zealand and Repell from the USA were known to have a high endophyte content and were included as 'controls'. The cultivars used were: Bastion, Belfort, Condesa, Compas, Cropper (2 seed lots), Ellett, Fantoom, Frances (2 seed lots), Kent Indigenous, Lidura, Magella, Melle, Meltra (2 seed lots), Morenne, Parcour, Repell, S24, Talbot (2 seed lots), Tove, Trani (2 seed lots). Three seeds of each seed lot were sown in potting compost in a plastic pot and the seedlings were grown on in a heated (c. 20°C) glasshouse. In May 1985 one tiller was removed from each pot and

a section of leaf sheath was examined for endophyte using the method described earlier.

(ii) Seed sown in field plots

In January and February 1985 samples of tillers were collected from plots of 38 cultivars of perennial ryegrass, which formed part of the National Institute of Agricultural Botany trials area at Seale-Hayne Agricultural College, Devon. The plots had been sown in 1983. The cultivars sampled were: Alsinto, Augusta (hybrid), Aurora, Barlenna, Borvi, Caradoc, Centurion, Challenger, Chieftain, Chevron, Compas, Conquest, Cropper, Devon Eaver, Enterprise, Fantoom, Flourish, Frances, Galliot, 'GFD/1', Gremie, Manawa, Melle, Meltra, Monta, Morenne, Pageant, Pennant, Perma, Plume, Reveille, RvP Hay Pasture, S24, Sperrin, Talbot, Tove, Trani, Wendy. Sections of leaf sheath were removed from five tillers of each cultivar and examined for endophyte using the method described above.

Experiment 3. Effect of endophyte on frit-fly larvae

Three cultivars were used in this study: Italian ryegrass cv. RvP, perennial ryegrass cv. Ellett, and a perennial ryegrass cultivar coded YR 843 (Yates Research, New Zealand). Ellett and YR 843 were known to have a high endophyte content, and RvP was included as a control because it is highly susceptible to attack by frit fly. Seed of the three cultivars was treated with the fungicide prochloraz (Sportak 20% SD; FBC Limited) at a rate of 12.5 ml product/kg seed. Treated and untreated seed was sown in potting compost in pots on 18 April 1985, and the pots were kept in a glasshouse before placing them in the field on 7 May 1985, when the seedlings had four or five leaves. Two sites at Hurley were used and at each site five pots of seedlings grown from untreated seed were put out for each of the three cultivars. On 5 June 1985 the seedlings were cut off at soil level and all tillers examined for frit fly larvae. Tillers containing larvae were examined for endophyte mycelium using the method described earlier.

RESULTS

Experiment 1, Survey of old grassland

Endophyte mycelium was detected in plants from eight of the 16 sites; these are shown on Fig. 1 as numbers 1, 4, 7, 9, 12, 13, 15 and 16.

The characteristics of infection were long strands of hyphae running intra-cellularly along the longitudinal axis of the leaf sheath, with little cross-branching. Sometimes the hyphae were convoluted. Morphologically the mycelium appeared identical to that observed in plants grown from seed from New Zealand known to be infected with Acremonium loliae.

Experiment 2. Commercial seed lots

In the seedlings grown in the glasshouse, endophyte was detected in the cultivars Ellett and Repell, the seed of which was known to have a high endophyte content, but no endophyte was found in the other cultivars.

Of the 38 cultivars examined from the field plots, endophyte was detected in only one, S24.

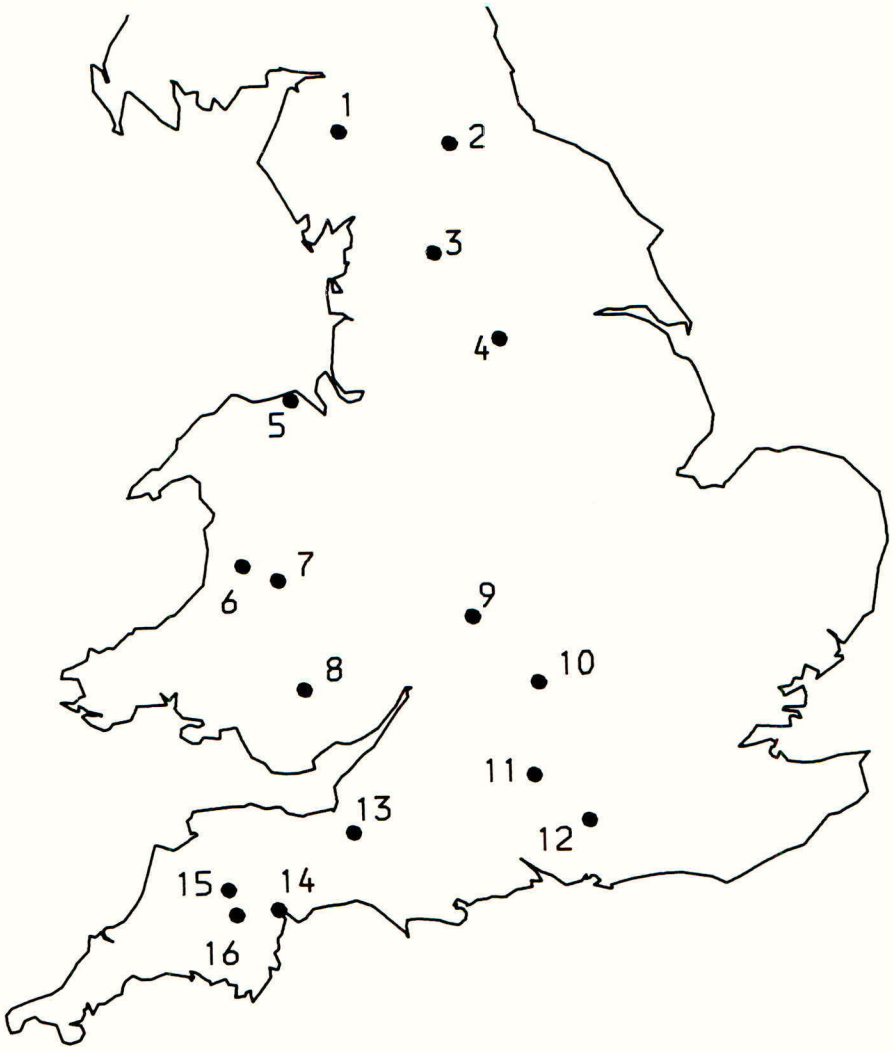

Fig. 1. Map showing locations of old pasture sites surveyed for endophyte.

Key

1. Penrith, Cumbria.
2. Barnard Castle, Durham.
3. Skipton, N. Yorks.
4. Barnsley, S. Yorks.
5. St. Asaph, Clwyd.
6. Ponterwyd, Dyfed.
7. Pant-y-dwr, Powys.
8. Tredegar, Gwent.
9. Great Alne, Warks.
10. Yarnton, Oxon.
11. Highclere, Hants.
12. Selborne, Hants.
13. Bridgwater, Somerset.
14. Exminster, Devon.
15. North Wyke, Devon.
16. Chagford, Devon.

Experiment 3. Effect of endophyte on frit fly

Frit fly larvae were found in all but one of the 60 pots placed in the field. Presence of endophyte in Ellett and YR 843 had no effect on larval numbers. A comparison between infected and non-infected plants was made possible because the fungicide seed treatment effectively eradicated endophyte infection (Table 1).

TABLE 1

Presence of frit fly larvae and endophyte mycelium in plants grown from endophyte infected seed and from seed treated with prochloraz fungicide. Plants placed in field for four weeks

Cultivar	% tillers with larvae		% tillers with endophyte	
	Untreated seed	Treated seed	Untreated seed	Treated seed
Site 1				
RvP	3.8	6.8	-	-
Ellett	2.3	7.5	-	5.6
YR 843	5.4	6.4	80.8	0
Site 2				
RvP	2.3	2.9	-	-
Ellett	4.4	3.3	71.9	0
YR 843	2.3	3.5	56.2	4.2

DISCUSSION

Endophyte infection of ryegrass plants in England and Wales appears to be widespread, in old pasture at least. Further sampling is in progress over a wide area, including Scotland. Also tests are in progress to determine whether the endophyte present is Acremonium loliae, although this is almost certainly the case (R.A. Prestidge, personal communication).

No endophyte was detected in ryegrass plants grown from seed currently available to farmers in Britain, with the exception of cv. S24, which may be relevant in that this is a long established 'landrace' cultivar. Although many cultivars were tested, the amount of seed sampled was very small, but it seems likely that if endophyte is present in modern cultivars it is at a very low level. In contrast, a high proportion of seedlings of the cultivars known to have endophyte infection were shown to be infected.

The preliminary test to confront frit fly larvae with endophyte infected plants gave disappointing results, since the fungus appeared to have no effect on infestation. However, it is

possible that a higher level of endophyte infection is needed, or an indigenous strain of endophyte rather than one from New Zealand, where the species of insect pests differ from those in Britain.

It was confirmed that treating seed with prochloraz is an effective means of removing endophyte infection.

ACKNOWLEDGEMENT

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CONTROL OF LEAF NEMATODES IN NUCLEAR STOCK STRAWBERRIES

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ABSTRACT

An infestation of leaf nematodes was discovered in Nuclear Stock strawberries. The successful measures taken to eradicate the pest and the changes in the scheme to prevent further similar infestations are described.

INTRODUCTION

Nuclear Stock strawberries, which are the basis of the British strawberry certification scheme, are kept at East Malling Research Station as two plants of each of approximately 130 varieties. These varieties were obtained directly from the original breeder to ensure, as far as possible, trueness-to-type and were tested for freedom from virus and fungal diseases prior to acceptance. They are maintained under conditions of strict sanitation and are continually monitored for disease (Ebbels 1979). Each year up to 20 varieties are requested by the Nuclear Stock Association to be multiplied by vegetative propagation (runners) to produce up to 300 plants per variety; these progeny are multiplied in successive years by selected nurserymen under constant supervision by MAFF inspectors responsible for granting certificates of health status.

The strawberry leaf nematode, Aphelenchoides fragariae, was accidently introduced into the stocks as a low-level infestation on an imported European variety. This species is widespread in strawberries in continental Europe where repeated treatment with the granular nematicide, aldicarb, prevents serious damage but does not eliminate the pest (Hirling 1969, Szczygiel 1970, Heide 1977). Although it occurs in a range of wild plants in Britain, it is rarely found in strawberries in this country, unlike the closely related species A. ritzema-bosi. The nematode remained unsuspected for several years and was only detected when plants were grown in the field and dramatic symptoms were observed; by which time it had spread to most of the other varieties in the scheme. As a result of this infestation the scheme was suspended for two years and no strawberry plants were released to the industry.

In retrospect it is obvious that this breakdown of the certification scheme occurred because of a failure to understand the host/parasite relationships of A. fragariae on strawberry. It had been assumed that this species would behave in a similar manner to A. ritzema-bosi which readily multiplies and produces marked leaf symptoms on potted strawberry plants in the glasshouse. The nematodes of both species live deep in the crowns of the plants feeding on the young, still-folded leaves; the damage to these leaves is later manifested, when they grow out, by severe distortion and obvious feeding areas. The symptoms produced by the two species are broadly similar (Böhmer 1979). It was, therefore, expected

that if nematodes were present they could be detected by the occurrence of symptoms. The only nematode check in the scheme had been observation of the stocks and testing of plants with suspected symptoms. It was subsequently discovered, however, that A. fragariae differs in requiring a period of increasing temperatures (equivalent to late winter/early spring) for multiplication and symptom expression. At constant glasshouse temperatures they are restricted to very low population levels, sufficient only for survival and occasional contamination of adjacent plants.

MATERIALS, METHODS AND RESULTS

The currently available nematode control measures, chemical and hot-water treatment, although highly efficient did not provide the 100% control essential for nuclear stock plants, each of which may be propagated further to produce a multiplication of several thousand times in subsequent years. Furthermore, it was difficult to establish the extent of the infestation because the testing method necessarily requires the destruction of the crown of the plant; it was possible to affirm that a plant was free of nematodes only after its destruction. A programme of control measures was, therefore, devised which incorporated a range of methods likely to produce nematode-free plants in the shortest possible time. This programme began with the meristem-culture of all the varieties, a technique which, although likely to produce many infested lines, still provided a chance of at least 30% of plants being free of nematodes. When the cultures had developed into mature plants they were immersed in water at 46°C for 10 minutes. Later in the season they were treated with aldicarb 'Temik 10G' at 1g per plant. The treated plants and their runner progeny were then kept in isolation from other lines and were subjected to a programme of sampling and roguing until it could be established beyond any reasonable doubt that a varietal clone was free of nematodes. The nuclear stock scheme could then be recommenced with most of the major commercial varieties.

Research has been continuing to find alternative, and less time-consuming nematode eradication measures suitable for nuclear stock plants. Several methods have been tested which have shown promise under certain specialised conditions but which were found to be unsuitable for large-scale application. A technique has recently been developed (McNamara, unpublished) and is now under test which seems to provide total eradication of leaf nematodes; it involves excision of those parts of the plant which may harbour nematodes, and subsequent regeneration of crowns from previously dormant buds.

DISCUSSION

The problems posed by this infestation illustrate the difficulty of protecting a vegetative propagation scheme from all possible pests and diseases, and show how vulnerable a single-source scheme can be, no matter how desirable for other reasons. The need for the highest standards of phytosanitation throughout is highlighted by the difficulty of eliminating this pest once established. The scheme has been considerably modified in the light of this infestation: all introduced varieties are now tested for nematodes and may receive a precautionary control treatment; mother plants

and a proportion of the progeny are regularly destructively tested for nematodes; plants are now grown in, and runners rooted into an artificial medium ('Perlite') instead of the previously-used peat from which it had been found difficult to eliminate all nematodes.

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VARIABILITY IN THE SAMPLING AND EXTRACTION PROCEDURES USED FOR THE DETECTION OF VIRUS-VECTOR NEMATODES

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ABSTRACT

Different sampling and extraction procedures are used in nematology laboratories in Britain for detecting virus-vector nematodes. It is suggested that some procedures are unsuitable for the detection of small populations of these nematodes and they may be particularly important if the nematodes are viruliferous especially if certification and statutory plant health schemes are involved.

INTRODUCTION

In Britain six soil-borne viruses are transmitted by nematodes, four by species of longidorids. Arabis mosaic and strawberry latent ringspot viruses are transmitted by Xiphinema diversicaudatum, raspberry ringspot virus by Longidorus elongatus and L. macrosoma and tomato black ring virus by L. elongatus and L. attenuatus. Two other viruses, tobacco rattle and pea early-browning, are transmitted by several species of Paratrichodorus and Trichodorus (Taylor 1978, Trudgill et al. 1983).

As part of the requirements of various fruit and hop certification schemes supervised by the Ministry of Agriculture, Fisheries and Food (MAFF) in England and Wales and a narcissi scheme in Scotland supervised by the Department of Agriculture and Fisheries for Scotland (DAFS) prospective sites are sampled for virus-vector nematodes. The Agricultural Development and Advisory Service (ADAS) for England and Wales and the Scottish Colleges of Agriculture Advisory Service for Scotland also examine soil samples for virus-vector nematodes. Jones et al. (1985) reported an increase in the incidence of some diseases caused by nematode transmitted viruses in commercially grown raspberry plantations and this may result in an increase in the numbers of soil samples submitted for examination by the advisory services for virus-vector nematodes.

The preliminary results of a review and an examination of procedures used in Britain to collect and subsequently to recover nematodes from soil samples taken to detect virus-vector species for certification and advisory purposes are presented here.

RESULTS AND DISCUSSION

Field sampling

From 1976 to 1980 almost 13,000 soil samples were examined by ADAS for migratory nematodes including the virus-vector species (Tab. 1; Mathias 1981). Fortytwo % of these samples were for advisory and diagnostic purposes whereas only 4.5% were for certification schemes, mostly in the south-east ADAS region.

The sampling tool used and the method of sampling differed between operators and the choice was influenced by the nematode genera being investigated. When samples are collected in England and Wales for certification purposes a standard sampling tool and sampling procedure is used (Cotten 1979). Some of the theoretical aspects of soil sampling for virus-vector

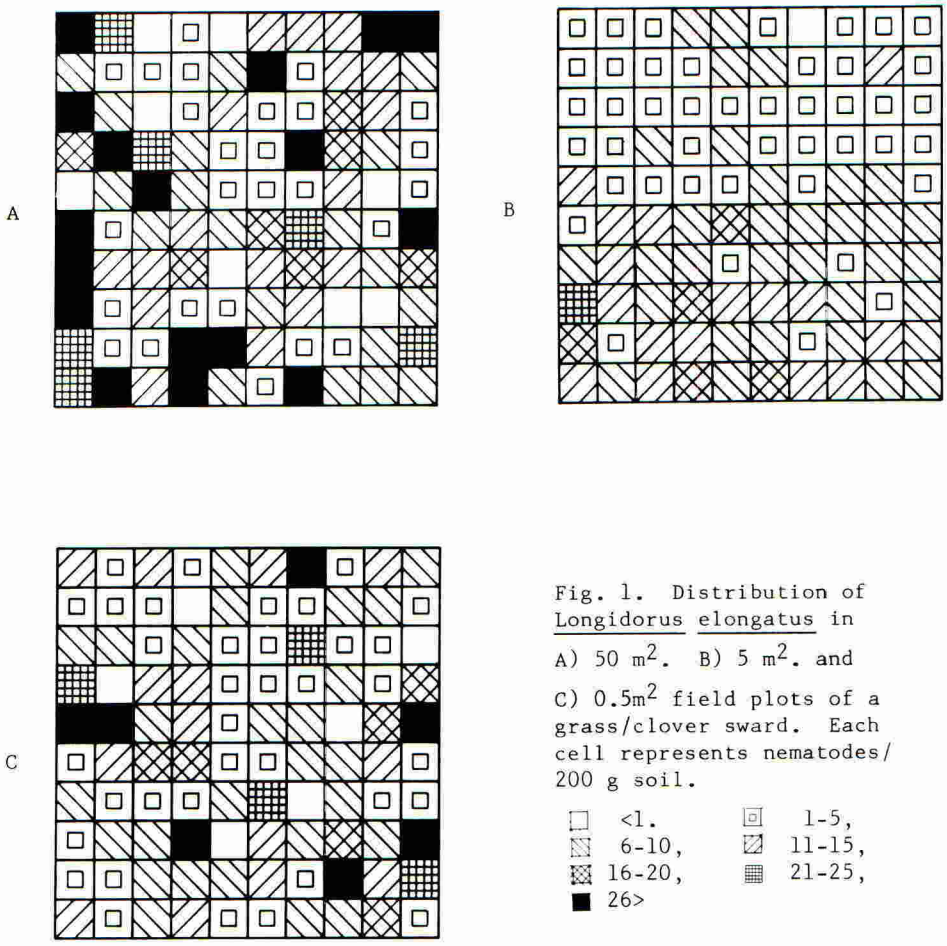

Fig. 1. Distribution of Longidorus elongatus in A) 50 m^2. B) 5 m^2. and C) 0.5m^2 field plots of a grass/clover sward. Each cell represents nematodes/ 200 g soil.

<1. 1-5,
6-10, 11-15,
16-20, 21-25,
26>

nematodes were presented by Cotten (1979) who also listed the probability, when using a 400 cm^3 sample size, of detecting nematodes randomly distributed in the soil. Boag & Topham (1985a) have shown that virus-vector nematodes do not have random distributions but that they are aggregated and that the distance between sampling points can influence their detection. The relative distributions of L. elongatus in field plots 50, 5 and 0.5 m^2 are shown in Fig. 1 and plot 1C is the top left square of plot 1B which in turn is the top left square of plot 1A. These distributions exemplify the need for a thorough sampling procedure. From the data in Fig. 1A it was calculated that to obtain an estimate of the mean population size, with a 90% accuracy, a composite sample was necessary of nine cores, each core being 5 cm dia. and 7 cm long. The practical aspects which have to be considered when sampling for virus-vector nematodes have been reviewed by Boag (1985). Indirect methods such as using the known associations between other nematode species and virus-vector nematodes (Boag & Topham 1985b) may also be useful for anticipating the occurrence of small populations of virus-vector nematodes.

Extraction of nematodes

Many methods are available for extracting migratory nematodes from soil samples (Southey 1970) but only five are commonly used in Britain (Tab. 2; Mathias 1981). Flegg's (1967) method (usually modified by the operator) is most widely used for extracting virus-vector nematodes although in a few laboratories a two flask method is used for trichodorids.

TABLE 1

The mean percentages of soil samples examined during 1976 to 1980 for migratory nematodes in six ADAS regions (Mathias 1981)

ADAS Region	Mean percentages of samples 1976 to 1980			Samples total
	Certification Schemes	Advisory and diagnostic	Investigational	
North	0.05	49	51	3691
Midlands	3	44	53	2282
East	4	45	51	4901
South-east	20	45	35	1119
South-west	0	17	83	491
Wales	0	53	47	223

After extraction the nematode suspensions are subjected to a cleaning and separating procedure, usually lasting 24 h. For longidorids, sixteen of the twenty-three laboratories listed in Tab. 2 used 92 µm-aperture plastic sieves, five used sieves ranging in aperture from 95 µm to 212 µm and the other centres used milk-filters. Fourteen of the laboratories used the Baermann funnel and nine the Petrie dish techniques for final separation of longidorids from soil debris. For trichodorid nematodes the techniques were more varied but most laboratories used a fiter supported on a plastic sieve; the filters ranging from 2 ply "Kleenex" tissues to various filter papers and single and double milk-filters. In two laboratories nematodes retained on 53 µm-aperture sieves were poured directly onto 92 µm-aperture final separating sieves and in a third laboratory onto a 105 µm aperture final separating sieve. In these latter three laboratories filters were not used.

TABLE 2

The five methods regularly used in Britain to extract migratory nematodes and the number of ADAS and other laboratories in which they are used (Mathias 1981).

Laboratory	n	Methods				
		W-H tray[1]	Flegg S/D[2]	Seinhorst two-flask	Seinhorst elutriator	Oostenbrink elutriator
ADAS	11	7	11	8	2	2
Others	12	5	12	4	1	1

[1]Whitehead-Hemming tray
[2]Flegg's modification of Cobb's sieving and decanting method

The MAFF Agricultural Science Service (Entomology) and ADAS Migratory Nematodes Working Party held an exercise in which five operators working in different laboratories collaborated in extracting Longidorus and Xiphinema nematodes from four different soils (Tab. 3; Cotten 1973). Four bulks of field soils were each thoroughly mixed, put into numbered polythene bags and allocated at random to operators. Each operator received five bags of each soil, each bag containing sufficient soil to enable 200 ml to be measured by displacement in water. The methods used by each operator were those used in their respective laboratories and were modifications of Flegg's (1967) method.

TABLE 3

The number of virus-vector longidorids recovered from four soils[1] by five operators each using Flegg's modification of Cobb's sieving and decanting method (Cotten et al. 1973)

Operator	Nematodes and numbers recovered			
	Longidorus attenuatus	L. elongatus	L. macrosoma	Xiphinema diversicaudatum
A	3.0[2] (2-4)	14.4 (6-20)	128.4 (107-149)	151.4 (99-195)
B	2.6 (1-5)	29.4 (15-42)	195.6 (172-236)	141.6 (122-157)
C	2.0 (1-4)	35.2 (24-53)	80.6 (56-102)	35.0 (25-52)
D	5.8 (4-7)	30.6 (24-38)	176.0 (160-194)	54.6 (33-97)
E	5.0 (2-9)	90.4 (65-119)	117.0 (106-121)	90.6 (64-117)
Variance ratio (4 degrees of freedom)	4.6	24.6	36.1	20.9

[1]Each soil contained only one virus-vector species and they were: organic soil with L. elongatus; sandy loam with L. attenuatus; fine sandy loam with L. macrosoma and clay loam with flints with X. diversicaudatum.

[2]Mean with minimum and maximum in parenthesis.

No individual operator was consistently effective with all soils and there were significant differences between operators in the results they obtained for each of the four soils. Recovery of X. diversicaudatum was particularly variable, the two smallest recoveries being only 23% and 36% respectively of the largest. The most variable recovery was with organic soil containing L. elongatus where the smallest recovery was only 10% of the greatest. With the exception of one operator the recovery of L. macrosoma was more consistent and although only small numbers of L. attenuatus were recovered by all operators the numbers still varied (Tab. 3).

A further experiment was done to assess the variation in results obtained by one operator when extracting nematodes from a series of samples taken from three thoroughly mixed bulks of field soils (Tab. 4). Ten 200 g

samples from each soil were extracted by modifications of Flegg's (1967) method; L. elongatus and X. index were extracted by one method which differed from that used for the trichodorids. The results were similar to those obtained by the Migratory Working Party. Differences between the largest and smallest recoveries for each species however were less than had been recorded in the previous study. The smallest recovery of trichodorids was 79% of the largest recovery whereas the smallest recoveries of L. elongatus and X. index were similar being 63% and 62% respectively of the largest recoveries with these nematodes.

TABLE 2

The number of nematodes recovered by one operator from ten samples from each of three thoroughly mixed bulks of soil[1].

Sample	Nematodes and the numbers recovered					
	Trichodorids			total	L. elongatus total	X. index total
1	20	100	210	330	95	284
2	40	100	200	340	99	315
3	90	40	220	350	98	461
4	40	90	190	320	107	336
5	10	80	240	330	125	364
6	30	100	250	380	93	461
7	60	50	190	300	79	430
8	50	80	240	370	91	351
9	50	60	190	300	100	417
10	30	60	240	330	103	398
Mean	42	76	217	335	99	382
Standard deviation	23	22	24	26	12	61
Coefficient of variation %	55	29	11	7.8	12	16

[1]The three soils were sandy loams and each soil contained only one of the three genera of nematodes

Some of the variability in the results obtained by the Migratory Nematode Working Party study (Cotten et al. 1973) probably was caused by differences in operator's skills in handling particular soil types. This especially may have influenced the results obtained with L. elongatus in a soil with a high organic content. Similar variations were evident when an operator extracted a series of samples using familiar extraction methods and soil types.

An awareness of the results presented here has resulted in workers in several laboratories in Scotland collaborating in a comprehensive study of sampling and extraction procedures and methods, used for detecting virus-vector nematodes. It is anticipated that the results of this study will enable a set of recommendations to be prepared and followed when dealing with virus-vector nematodes and their associated viruses (M.M. MacKenzie pers. comm).

CONCLUSIONS

Most virus-vector species in Britain are relatively efficient at transmitting their associated viruses (Trudgill et al. 1981) and their detection is important even when present in small numbers. From the results presented here it is likely that small population levels may go undetected as a result of inadequate sampling of field sites and/or inefficient extraction techniques having been used. Also, the absence of standard procedures for sampling and extracting virus-vector nematodes provides an opportunity for differences to exist within Britain in the standards adopted for certification and for statutory purposes.

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EFFICIENCY OF SOME DISINFECTANTS CHALLENGED BY A RANGE OF PLANT PATHOGENIC BACTERIA

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ABSTRACT

In vitro disinfectant tests were performed with 6 representative isolates from 4 bacterial genera: *Clavibacter* (*Corynebacterium*) *michiganense*, *Erwinia carotovora*, *Pseudomonas corrugata*, *Pseudomonas marginalis*, *Xanthomonas campestris* and *Xanthomonas graminis*. Twenty-two compounds belonging to the chemical groups alcohols, aldehydes, hypochlorites, metals, phenols, quaternary ammonium compounds and organic peracids were screened. Two phenols, Clearsol and Sudol, two quaternary ammonium compounds, benzalkonium chloride and N-cetylpyridinium chloride, at concentrations of 10%, and Bronopol, at 1%, were bactericidal against all 6 of the pathogens within a 15s contact time under "dirty" conditions.

INTRODUCTION

Clavibacter (*Corynebacterium*) *michiganense* subsp. *michiganense* (bacterial canker), *Erwinia carotovora* var. *carotovora* (soft rot), *Pseudomonas corrugata* (tomato pith necrosis), *Pseudomonas marginalis* (soft rot), *Xanthomonas campestris* pv. *campestris* (black rot) and *Xanthomonas campestris* pv. *graminis* (bacterial wilt) are examples from 4 bacterial genera containing plant pathogenic types. They affect a variety of crops and ornamental plants, and have in common the ability to be spread through wounding and cultivational practices such as pruning, grafting and the taking of cuttings (C.M.I. Descriptions of pathogenic Fungi and Bacteria). For all of these diseases, the main methods of control are through crop rotation, hygiene and the production of resistant varieties. Therefore disinfectants play an important role in disease management.

Previous disinfectant work has been done with compounds such as mercuric chloride against the bacterium *C. sepedonicum* (Lane *et al* 1948) and formaldehyde as a successful disinfectant for *P. solanacearum* (Buddenhagen & Sequeira 1958). Sodium hypochlorite is an inhibitor of *P. tolaasii*, (Ayers & Lambert 1955, Wong & Preece 1985a), *P. syringae*, *P. viridiflava*, *E. chrysanthemi*, *E. herbicola* (Palazon *et al* 1981) *E. amylovora* (Keil & van der Zwet 1967) and *X. pelargonii* (McPherson 1981). Bronopol is bactericidal against *P. tolaasii* (Wong & Preece 1985b,c) and *E. salicis* (Amels 1981) and British Patent No. 1057 131 also claims activity against *X. malvacearum* and *E. amylovora*.

In this paper an attempt has been made to screen as many disinfectants

as possible against a range of plant pathogens, so that advice to growers on knife-dipping, foot baths, handwashing and glasshouse disinfection can be given on the basis of work done with more than one pathogen.

MATERIALS AND METHODS

Organisms

Isolates of Clavibacter (Corynebacterium) michiganense subsp. michiganense NCPPB 1468, Erwinia carotovora var. carotovora NCPPB 312, Pseudomonas corrugata NCPPB 2457, Pseudomonas marginalis NCPPB 247, Xanthomonas campestris pv. campestris NCPPB 2517 and Xanthomonas campestris pv. graminis NCPPB 2700, were obtained from the National Collection of Plant Pathogenic Bacteria, Hatching Green, Harpenden, Herts. They were prepared by suspending the growth from a 48 hour culture in sterile distilled water.

Disinfectants

The disinfectants tested, with their percentage active ingredients (where supplied) were as follows: Applied 3-78, 4-chlorophenol (Applied Chemicals Ltd,), benzalkonium chloride, 50% alkyldimethylbenzylammonium chloride (Koch Light Laboratories Ltd.), Bronopol, 98-100% 2-bromo-2-nitro-propane-1,3 diol (Boots Biocides Ltd.), N-cetylpyridinium chloride (B.D.H. Chemicals Ltd.), Clearsol, 40% xylenol (Tenneco Organics Ltd.), Dettol, 4.8% chloroxylenol (Reckitt and Colman U.K. Products Ltd.), Dettox, 1.25% quaternary ammonium (Reckitt and Colman U.K. Products Ltd.), Environ, phenolic mixture (Darmycel U.K.), Ethanol, absolute (B.D.H), formaldehyde, 40% (B.D.H.), H48, 84% magnesium monoperoxyphthalatehexahydrate (Interox Chemicals Ltd.), Hycolin, 15% phenolics (William Pearson Ltd.), Izal, 1.25% di-chloroxylenol, 1.25% chlorolene, 4.5% terpenes (Sterling Health U.K.), Jeyes Fluid, 20% soluble coal tar acids (Jeyes Ltd.), Kohrsolin, acetale glutaraldehyde (Bode Laboratories U.K.), Panacide M, 40% G-4,5,5-dichloro-2-2-dihydroxydiphenylmethane (B.D.H.), Parozone bleach, sodium hypochlorite (Jeyes Ltd.), Savlon, 3% cetrimide, 0.3% chlorhexidine gluconate (I.C.I. plc), Sudol, 50% xylenol mixture, (Tenneco Organics Ltd.), Triple Fresh, quaternary ammonium compound, (Applied Chemicals Ltd.), Vitigran, 50% copper oxychloride (Hoescht U.K. Ltd.) and zinc omadine, 48% cyclic thiohydroxamic acid (I.C.I. - Midox Chemicals plc).

Suspension tests

The disinfectants were serially diluted using sterile distilled water to give 0.1, 1 and 10% weight/volume or volume/volume of the formulation. An aliquot of 100µl of the bacterial suspension containing 10^8 cells was added to 9ml of the diluted chemical, and after 15, 30 and 60 seconds, 10µl of the solution were transferred to tubes containing 5ml nutrient broth (Difco). These tubes were then incubated at 25°C for 3 to 7 days and presence or absence of growth recorded. The most effective chemicals were then re-tested with 10% w/v sterile peat added ("dirty conditions").

RESULTS

All of the chemicals tested showed disifectant activity against at least 1 of the 6 pathogens. The bactericidal properties of Dettol(phenol), Dettox (Q.A.C.), Parozone (hypochlorite) and Savlon (Q.A.C./biguanide) were affected by the presence of peat, while those chemicals least affected were: Clearsol

TABLE 1

Bactericidal effectiveness of 22 chemicals against 6 bacterial plant pathogens, with a contact time of 15s, in the presence of 10% w/v sterile peat.

Chemical Family	Compound	Effective chemical concentration (%)+ Pathogens* 1468 C.m.	312 E.c.	2457 P.c.	247 P.m.	2517 X.c.	2700 X.g.
Alcohols	Ethanol	-	-	-	-	-	-
	Bronopol	1	1	1	1	1	0.1
Aldehydes	Formaldehyde	-	-	10	10	10	1
	Kohrsolin	10	-	-	-	10	10
Hypochlorite	Parazone	-	1	-	1	10	-
Metals	Vitigran	-	-	-	-	-	10
	Zinc omadine	10	-	10	-	-	1
Phenols	Applied 3-78	10	10	-	-	-	-
	Clearsol	10	1	10	1	1	10
	Dettol	10	10	-	-	-	-
	Environ	-	1	-	10	1	1
	Hycolin	-	1	10	10	1	10
	Izal	10	-	-	-	10	10
	Jeyes Fluid	10	10	10	-	1	1
	Panacide	0.1	10	-	1	1	1
	Sudol	1	1	10	1	1	1
Q.A.C's	Benzalkonium chloride	0.1	10	1	1	0.1	0.1
	N-cetylpyridinium chloride	0.1	10	1	10	0.1	10
	Dettox	10	-	-	-	10	10
	Triple Fresh	10	-	-	-	10	10
Q.A.C./ biguanide	Savlon	-	10	10	-	10	10
Organic peracid	H48	-	0.1	1	10	1	10

+ % is in terms of w/v or v/v of the formulation

* C.m.=C.michiganense, E.c.= E.carotovora, P.c.= P.corrugata, P.m.= P. marginalis, X.c.= X.campestris, X.g.= X.graminis

Hycolin, Izal, Panacide and Sudol (phenols). Five chemicals: 10% benzalkonium chloride and N-cetylpyridinium chloride (Q.A.C.'s), 10% Clearsol and Sudol (phenols) and 1% Bronopol (alcohol) killed all of the pathogens within 15s in "dirty" conditions (see Table 1). A further four compounds killed five out of the six pathogens: 10% Hycolin, Jeyes Fluid and Panacide (phenols) and 10% H48 (organic peracid).

The easiest bacteria to kill were the xanthomonads, all but four or five chemicals were effective against them and the hardest to kill were the pseudomonads, where half of the compounds tested failed to kill these bacteria.

DISCUSSION

Only the results for disinfectants challenged with bacteria for the shortest time of 15s in "dirty" conditions are included in this paper as these results were felt to be the most relevant to the field situation. They also showed up best which were the most efficient of the 22 chemicals.

The use of phenolics is not without problems due to their skin irritant properties on contact. Bronopol and N-cetylpyridinium chloride were difficult to dissolve in water. Traditional disinfectants such as formaldehyde, which is noxious to use and sodium hypochlorite, which is corrosive, did not work against all of the pathogens. These compounds should not be recommended without a thorough investigation into individual plant pathogens. Further work is in progress to evaluate the most efficient compounds: Bronopol, Clearsol, Sudol, benzalkonium chloride, N-cetylpyridinium chloride, Hycolin, Jeyes Fluid Panacide and H48 against other plant pathogens including Agrobacterium sp.

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FF4050 - A FLUTRIAFOL AND ETHIRIMOL BASED SEED TREATMENT TO CONTROL BARLEY DISEASES

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ABSTRACT

FF4050 is a new systemic fungicidal seed treatment containing flutriafol, ethirimol and thiabendazole. Applied at the rate of 500 ml/100 kg seed it provides dual mode of action against barley powdery mildew (Erysiphe graminis). Trials have demonstrated good crop safety, whilst mildew control and yield increases have been superior to current commercial treatments.

INTRODUCTION

UK winter and spring barley crops are subject to heavy disease pressures, particularly as farmers strive for maximum yields through high fertiliser use, and earlier sowing of winter barley. In the absence of varieties with stable resistance, fungicide use is necessary to control diseases for optimum yield.

The most important disease of barley in the UK and Northern Europe is powdery mildew caused by Erysiphe graminis. Ethirimol was introduced in 1969, the first seed treatment for systemic control of cereal powdery mildew. After its introduction, powdery mildew populations were examined over a period of six years and showed reduced sensitivity to the fungicide in those crops where it had been used. Nevertheless mildew control and yield benefits justified its use throughout this period. Its use on winter barley was withdrawn for a period of four years to avoid additional selection pressure during the autumn and winter (Shephard et al. 1975). Use of ethirimol declined following the introduction of triadimenol/fuberidazole as a seed treatment in 1978. This triazole fungicide offered good control of seed- and soil-borne diseases in addition to systemic control of powdery mildew and other air-borne diseases. Following extensive use of triazole foliar sprays and triadimenol-based seed treatments since 1978, reductions in the sensitivity of powdery mildews to these fungicides have also been noted (Fletcher & Wolfe 1981).

The reduction in powdery mildew sensitivity to triazoles has been concomitant with an increase in sensitivity of the same populations to ethirimol (Heaney et al. 1984). However, triazole fungicides have maintained reasonable efficiency against cereal powdery mildews, and as seed treatments their broad spectrum of action against seed- and soil-borne disease remains a major justification for their use. Work in Scotland showed that ethirimol efficiency had improved there in recent seasons (Stoddart & Northwood 1984).

Generally it is accepted that disease control is less likely to be reduced through resistance when use is made of mixtures of compounds with differing modes of action. FF4050 is such a mixture. It is a liquid formulation containing 30g flutriafol, 400g ethirimol and 10g thiabendazole per litre. The recommended application rate is 500ml per 100kg of seed.

Skidmore et al. 1983, described the control with flutriafol of all the important seed, soil and air-borne diseases of barley - loose smut (Ustilago nuda), covered smut (Ustilago hordei), seedling blight and foot rot (Fusarium spp), leaf stripe (Pyrenophora graminea), seed-borne net blotch (Pyrenophora teres), powdery mildew (Erysiphe graminis hordei), leaf blotch (Rhynchosporium secalis), yellow rust (Puccinia striiformis) and brown rust (Puccinia hordei). This paper describes laboratory and field studies which evaluated powdery mildew control and crop safety using FF4050 compared with ethirimol (+ mercury) and the standard triadimenol + fuberidazole seed treatment.

MATERIALS AND METHODS

FF4050 was tested from 1982 onwards on winter and spring barley. All trials were fully replicated using randomised block designs and were conducted throughout the main cereal growing areas of the UK. Plot sizes were 72 to 120 m^2. Crop safety was assessed in the field and laboratory. Crop emergence and establishment were evaluated by randomly selecting five 1 m row lengths per plot and counting the number of normal plants. The standard MAFF germination test was also used, where four replicates each of 100 seeds were sown in Levington Universal compost at a depth of 15mm in seed trays, watered, covered, and kept at a constant 20°C for 6 days. Tests were assessed by a qualified seed analyst for the percentage of normal seedlings.

Disease assessments were made according to recognised guidelines on 10 to 25 leaves per plot and the percentage area of leaf covered by disease recorded. Yield data presented is corrected to 15% moisture content. Trials were harvested using a small plot combine harvester and yields recorded. Where results from a single trial are reported, Duncan's multiple range test (Duncan 1955) was used to compare statistically each treatment mean; values followed by a common letter are not significantly different at $p = 0.05$.

Seed was treated by either a Rotostat or Centaur applicator. FF4050 (3% flutriafol/40% ethirimol/1% thiabendazole as wt/vol) was applied at 500ml/100kg of seed. Ethirimol was applied as 'Milstem' (58% wt/vol ethirimol) at 670 ml/100kg of seed. Triadimenol + fuberidazole was applied as 'Baytan' (25% triadimenol/3% fuberidazole wt/vol) at 150g/100kg of seed. Triadimenol + fuberidazole + imazalil was applied as 'Baytan' IM (25% triadimenol/3% fuberidazole/3.3% imazalil wt/vol) at 150 g/100 kg seed. Mercury was applied as 'Ceresol' (2% wt/vol phenyl mercury acetate) at 110ml/100kg of seed.

Details of the locations of field trials, barley varieties and drilling dates are shown below:

Trial No	Location	Variety	Drilling Date
EA 1/82	Walsham-le-Willows, Suffolk	Georgie	27.3.82
EA 2/82	Rede, Suffolk	Georgie	26.3.82
NE 2/82	Dorrington, Lincs	Koru	28.3.82
NA 21/84	Balsham, Cambs	Koru	07.3.84
EA 12/84	Norton, Suffolk	Georgie	16.3.84
SW 22/84	Tetbury, Glos	Georgie	07.3.84
EM 2/85	Yaxley, Cambs	Igri	25.9.84
EM 4/85	Ickleton, Cambs	Maris Otter	10.10.84
NA 3/85	Balsham, Cambs	Maris Otter	20.9.84
NE 5/85	Kettlethorpe, Lincs	Igri	04.10.84
WM 6/85	Kineton, Warwicks	Igri	15.10.84
EA 17/85	Haughley, Suffolk	Goldmarker	05.4.85
SW 19/85	Crewkerne, Somerset	Triumph	12.3.85
WM 11/85	Butlers Marston, Warwicks	Koru	15.4.85

RESULTS

Seed germination figures are presented in Table 1 for a range of important winter and spring barley varieties. FF4050 was safe with all varieties giving greater than the minimum 85% normal germination required by the Cereal Seed Regulations, 1980.

TABLE 1

Germination of treated winter and spring barley varieties (%)

Winter Barleys	Gerbel	Igri	Pirate	Tipper	Maris Otter
Untreated	98	98	98	97	98
FF4050	99	93	97	97	96
Triadimenol/fuberidazole	98	92	98	95	97
Spring Barleys	**Kym**	**Atem**	**Patty**	**Triumph**	**Golden Promise**
Untreated	94	98	93	98	98
FF4050	94	93	90	96	96
Triadimenol/fuberidazole	96	94	89	99	98

Table 2 presents the mean crop emergence and establishment figures from trials in 1982, 1983 and 1984 on winter and spring barley. FF4050 and the triadimenol/fuberidazole mixture caused a slight reduction in speed of emergence but eventual crop establishment was good.

TABLE 2

Crop emergence and establishment as % of mercury standard, 1982-84

Crop	Winter Barley				Spring Barley			
Year	1982	1983	1982	1983	1983	1984	1983	1984
Assessment	emergence		establishment		emergence		establishment	
No. of Trials	6	9	6	9	9	10	9	10
Mercury (Hg)	100	100	100	100	100	100	100	100
FF4050	84	88	91	95	89	87	97	99
Triad/fub'zole	84	89	90	96	90	93	95	99

Powdery mildew control after thirteen weeks in 1982 and 1984 from six trials on spring barley is presented in Table 3. Overall FF4050 was superior to the other treatments.

In 1984 FF4050 and ethirimol gave high levels of mildew control and were both superior to triadimenol/fuberidazole at eight and thirteen weeks after drilling (Northwood et al. 1984). This difference was evident in eight trials out of ten in this series.

TABLE 3

Mildew control (%), spring barley, 1982 and 1984 - 13 weeks after drilling

Year	1982	1982	1982	1984	1984	1984
Trial No.	EA1	EA2	NE2	NA21	EA12	SW22
Untreated (Actual)	(32.4)a	(17.2)a	(40.0)a	(10.4)a	(16.7)a	(46.8)a
FF4050	94b	87c	96c	99c	93c	97c
Triad/fub'zole	92b	78b	88c	78b	58b	69b
Ethirimol (+Hg)	96b	52b	37b	97c	85c	97c
Leaf Assessed	L1	L2	L2	L4	L3	L2

Again, in 1985, FF4050 gave excellent mildew control, out-performing the standard in both level of control and persistence (Table 4).

TABLE 4

Mildew Control (%), spring barley, 1985

Trial No.	EA17		SW19		WM11	
Wks after drilling	8	13	10	12	14	16
Untreated (Actual)	(2.6)a	(45.9)a	(5.1)a	(11.6)a	(3.6)a	(15.3)a
FF4050	89c	92c	96c	80c	91c	86c
Triad/fub'zole	54b	42b	86b	57b	49b	36b
Leaf Assessed	L3	L3	L3	L2	L4	L2

Grain yields demonstrated the superiority of FF4050 compared to triadimenol/fuberidazole and ethirimol alone (Table 5).

TABLE 5

Yield as % untreated, 1982, 1984 and 1985 spring barley

Trial No.	EA1/82	NE2/82	NA21/84	EA12/84	EA17/85	SW19/85
Untreated (t/ha)	(5.13)a	(4.35)a	(5.44)a	(4.95)a	(4.45)a	(5.13)a
FF4050	128c	118b	115b	126c	109c	127c
Triad/fub'zole	120b	113b	110b	110b	101b	111b
Ethirimol (+Hg)	120b	104a	114b	122c	-	-

Autumn mildew control on winter barley was also superior with FF4050 compared to triadimenol/fuberidazole/imazalil (Table 6). FF4050 can have a marked beneficial growth effect on this crop when the important autumn diseases are controlled. Increased plant size and tiller strength, together with a reduction in stem basal browning was observed. This allows the crop to better withstand adverse winter conditions and provides an excellent basis for early and rapid spring growth.

TABLE 6

Mildew control (%), winter barley, Autumn 1984

Trial No.	EM4	EM2	NA3	NE5	WM6
Wks after drilling	9	12	12	13	16
Untreated (Actual)	(26.2)a	(8.4)a	(29.3)a	(5.4)a	(12.9)a
FF4050	78c	69c	94c	69c	87c
Triad/fub'zole/imaz	49b	37b	52b	26b	54c
Leaf Assessed	L3	L3	L3	L3	L3

DISCUSSION

A major consideration in the development of a new seed treatment is crop safety. FF4050 was safe to all winter and spring varieties evaluated in laboratory germination and field tests over three years.

Field trials in 1982 demonstrated improved mildew control with the two triazole-based treatments compared to ethirimol. However, in 1984, control after thirteen weeks with ethirimol was particularly good in all three spring barley trials displayed (NA21, EA12, SW22) with high disease pressure. Also better mildew control with FF4050 compared to the triadimenol/fuberidazole/imazalil mixture was observed in 1984 and 1985 on both winter and spring barley. These effects may be attributable to the greater sensitivity of mildew populations to ethirimol in 1984 and 1985 than in 1982. Yield increases obtained from fungicide treatment correspond well with the levels of mildew control achieved.

Overall the results confirm that flutriafol with ethirimol forms the basis of an excellent product. It gave a more stable and better performance through time than either ethirimol or triadimenol/fuberidazole alone. This greater predictability of performance against mildew, the major disease of barley, in a situation of changing pathogen sensitivity, is of great value to the grower.

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THE SCOTTISH SEED POTATO CLASSIFICATION SCHEME AND THE PRODUCTION OF NUCLEUS STOCKS USING MICROPROPAGATION

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ABSTRACT

Scotland grows about 21 000 ha of seed potatoes. Statutory regulations ensure cultivar purity and limit the spread of pests and diseases. The Department of Agriculture and Fisheries for Scotland (DAFS) is responsible not only for enforcement of the regulations, but also for the production of nucleus stocks which are the starting point for most seed potato production in Great Britain. These stocks were grown originally from virus-tested stem-cuttings but micropropagation has now replaced this technique. Annually, 40 000 tubers harvested from microplants at DAFS nucleus stock farm are supplied to 48 specialist growers for clonal multiplication to VTSC, the highest seed potato class.

INTRODUCTION

The potato can be affected by many diseases caused mainly by viruses, fungi and bacteria which may lower yields by reducing photosynthetic efficiency and rotting the tubers (Hide & Lapwood 1978). Strategies for the production of healthy seed have developed as our knowledge of disease epidemiology has increased, and our perspectives and abilities to control disease has changed (Howell, these proceedings). Basic requirements include: a regular input of pathogen-tested nucleus stocks, together with control of the number of years succeeding crops are multiplied and methods to limit pest and disease re-introduction, possibly by restricting potato growing to certain areas because of, for example, the low incidence of aphid-borne viruses or absence of soil-borne pests (e.g. potato cyst nematode, PCN) or diseases (e.g. wart disease). These requirements are embodied in statutory regulations which relate to the Scottish Classification Scheme (Anon. 1984) and are enforced by the Department of Agriculture and Fisheries for Scotland (DAFS). They cover the production and marketing of seed potatoes and, in particular, define tolerances in respect to cultivar purity and pest and disease levels in the growing crop and harvested tubers. In many respects Scottish requirements are more stringent than those specified in EEC Directive 66/403/CEE which lays down minimum conditions for the marketing of seed potatoes in member states.

THE CLASSIFICATION SCHEME

The seed potato industry has developed in Scotland because of climatic conditions which limit the spread of aphid-borne virus diseases (Turl 1981). Scotland is a major world producer of seed potatoes and in 1984/85, from about 21 000 ha, exported 314 500 t valued in excess of £30m of which 231 000 t were sold to traditional markets in England and Wales. The importance of Scotland as the major seed producing area of the UK is recognised and, together with parts of Northern England, comprises the Protected Region (Anon. 1974) where imports of seed potatoes are banned except under licence and entry through the National and Breeders'

Quarantine Units (NQU and BQU) (Rose & Miller-Jones, these proceedings). In addition, only seed of the basic classes (VTSC - Virus-tested stem-cutting, SE - Super Elite and E -Elite) may be grown.

The Classification Scheme is based on the annual production by DAFS of nucleus stocks. These were originally derived from virus-tested stem-cuttings in an attempt to flush out tuber-borne diseases (Hirst & Hide 1967, Graham & Hardie 1971) which were particularly troublesome in the 1960s (Hay 1969). Since 1981 however, the stem-cutting technique has been replaced by micropropagation. Annually, 40,000 tubers grown from microplants at DAFS nucleus stock farm are distributed to 48 approved growers in the Protected Region for 3 years' clonal multiplication to VTSC, the highest seed potato class (Table 1). The right to remain a VTSC grower depends on reaching an acceptable performance at each annual crop inspection, and maintaining exceptional standards of crop husbandry which are outlined in the Code of Practice for VTSC Growers (Anon. 1985a) and following other rules which are designed to limit the spread of pests and diseases, such as the use of fungicides to control gangrene (Phoma exigua var. foveata) and skin spot (Polyscytalum pustulans).

TABLE 1

Visual tolerances at final field inspection (% plants).

	Seed classes			
Diseases/Disorders	VTSC	SE	E	AA
Tobacco veinal necrosis virus	0.00	0.00	0.00	0.25
Severe mosaic	0.00	0.00	0.10	
Leaf roll virus	0.00	0.01		
Mild mosaic	0.00	0.05	0.50	1.00
Blackleg (Erwinia carotovora subsp. atroseptica)	0.00	0.25	0.50	1.00
Purity and trueness to type	100.00	99.95	99.95	99.90

The pathway for entry into the Classification Scheme is shown in Fig. 1. By limiting the time that stocks may retain their VTSC classification to 2 years and SE to 3 years, a 'flushing out' of disease is assured. At present there is no generation control on E stocks. In 1985 the areas classified were as follows: VTSC 1 (89 ha), VTSC 2 (270), SE 1 (1526), SE 2 (4941), SE 3 (3597), E (6530), AA (1887). The AA class cannot be planted for further seed production in the Protected Region.

THE NUCLEUS STOCK PRODUCTION UNIT

In vitro work and disease indexing is done at East Craigs and glasshouse and field work at DAFS nucleus stock farm, Ingraston, which is situated in a livestock area of Peeblesshire, 30 km south west of Edinburgh. Experience over 16 years has shown that the location, and the

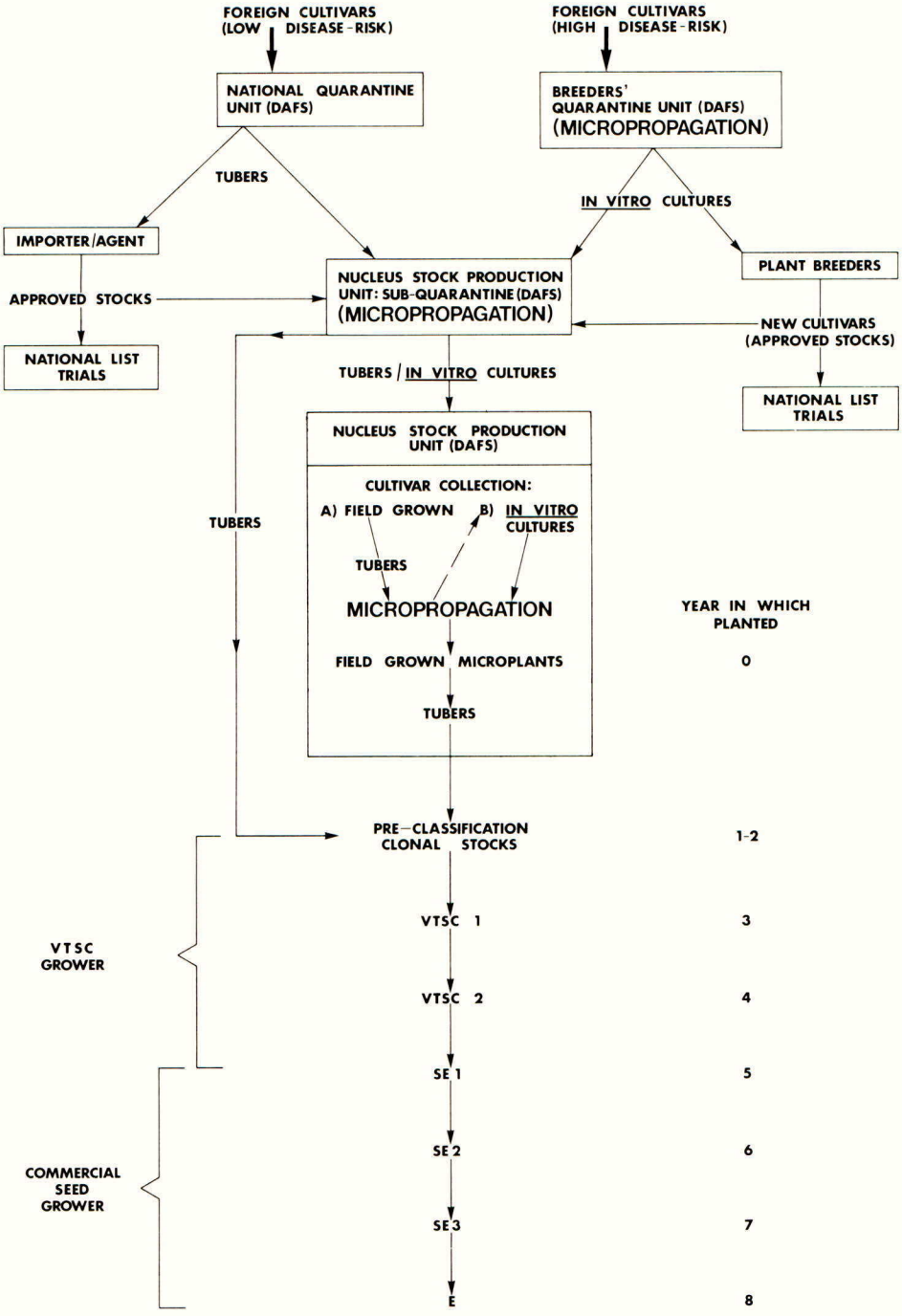

Fig.1. Pathway for the entry of cultivars into the Scottish Classification Scheme.

exceptional husbandry practices employed there, minimise the risk of the nucleus stocks becoming contaminated and infected by potato pathogens.

The Nucleus Stock Production Unit has its own sub-quarantine facilities near East Craigs where the initial disease indexing and multiplication of all new cultivars is performed. Usually these are submitted by Plant Breeders or their Agents from Approved Stocks (Anon. 1985b) at the start of National List Trials, and occasionally direct from the NQU or BQU (Fig. 1) if more rapid commercialisation is required. Microplant cultures are established while disease indexing of the tuber sap, eye-plugs and mother plant proceeds. Once the initial tests are completed the microplants are multiplied and planted in the glasshouse to produce tubers which can be released for pre-basic clonal multiplication.

Procedures

For use in the production of nucleus stocks, 180 pathogen-tested cultivars are maintained in the field and as *in vitro* microplant cultures (incubated at 6°C, 16 h photoperiod in a Vindon Scientific cooled incubator). In practice *in vitro* cultures are mostly used since they provide additional security over the field collection in terms of health. The production programme shown in Fig. 2 starts in December when orders are received from VTSC growers. Clones ordered in December 1985, for example, will be produced by DAFS in 1986 for planting by the VTSC grower in 1987.

The growth medium used for culture work is that of Murashige and Skoog without growth regulators (M&S) (Flow Laboratories; catalogue No. 26-100-20) but with 30 g/l sucrose and 8 or 5 g/l Oxoid No. 3 Agar. The lowest concentration of Agar is used only for the final rooting in Petri dishes. *In vitro* work is done in laminar flow cabinets using aseptic procedures. Cultures are incubated at 18° - 20°C, 16 h photoperiod (cool-white fluorescent tubes, 120-220 microeinsteins/m^2/sec at the bench surface).

To set up cultures, tuber sprouts (grown in the dark) are surface sterilised in 2.5% Deosan for 10 min and rinsed with one wash of sterile water before excision of the axillary buds which are 'planted' into M&S medium. Every 4-6 weeks, the resulting microplants are sub-divided into nodal segments and transferred to fresh medium. When the required multiplication has been obtained (we limit numbers to about 200 microplants from each tuber) the nodal segments are rooted in Petri dishes and then planted into peat blocks (Jiffy 7) under mist in the glasshouse.

After 3-4 weeks growth, the microplants are planted in the field at a 0.3 m spacing in furrows 1.4 m apart, in soil which has been tested for freedom from PCN and nematode transmitted viruses. Usually no planting is done before 1 June because of the risk of frost. Pellets to control leather jackets are applied to the soil surface and the plants earthed up during growth. The plants are regularly examined and blight control and aphicide sprays applied when necessary. Stocks are irrigated (with river water) to improve plant establishment, reduce yield variability, control common scab, and advance the harvest date thereby reducing the risk from aphid-borne viruses and pathogens borne in atmospheric aerosols (Carnegie 1980, Quinn *et al.* 1980). Since potato pathogens such as *Erwinia* spp. may be present in river water (McCarter-Zorner *et al.* 1984) it is sterilised by passage through an ultra-violet light reactor (Model AA60, Willand Process Plant Ltd, Wigan) at a flow rate of 13 000-15 000 l/h. Late maincrop

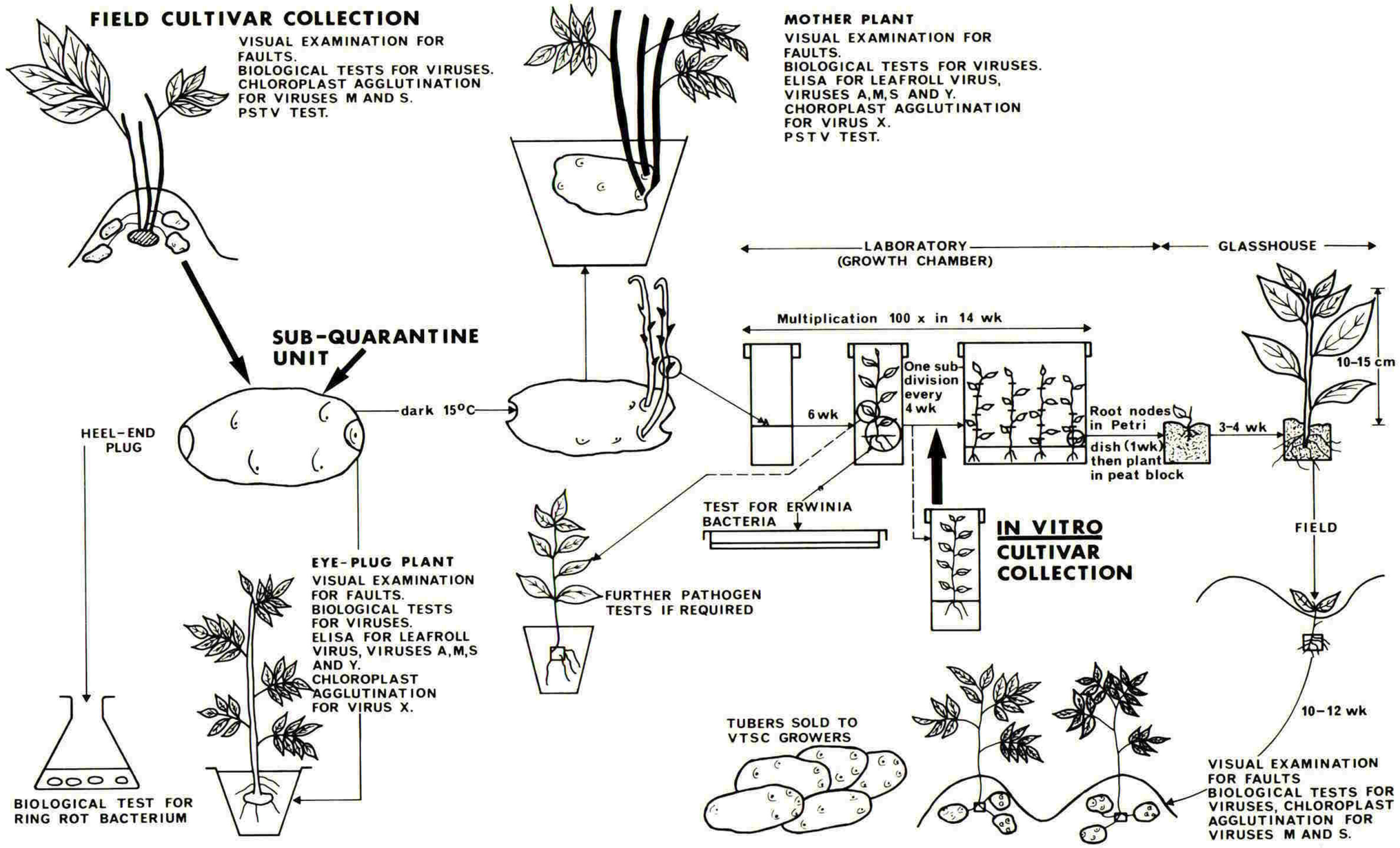

Fig. 2. Disease testing and production programme for potato nucleus stocks.

cultivars (e.g. Cara and Pink Fir Apple) may be planted initially under a mulch (Visqueen or Agronet) to advance the harvest date.

The plants are hand-dug as soon as the tubers are of seed-size and leaf samples collected for virus testing. To insure against contamination by skin spot and gangrene (Carnegie et al. 1981) the stocks are fumigated with 2-aminobutane (Graham et al. 1973). After careful selection into clones of 24 tubers, consignments are dispatched to the VTSC growers.

Disease Testing

In order to screen against a wide range of pathogens, tests are conducted several times during the production programme using various methods (Fig. 2). Additional tests are done on material at the sub-quarantine facility.

Viruses

Sap is expressed from leaf samples using a Pollähne sap extractor modified to give cold water flushing of plant debris from the rollers, hot water sterilisation and then cold water cooling (Laidlaw 1986). Presence of virus is tested by inoculation of sap onto susceptible indicator plants using specialised inoculation equipment to optimise the sensitivity of the test (Laidlaw 1985, 1986). The most commonly used indicator plant is Nicotiana tabacum cv. White Burley but, in testing mother plants, N. debneyi and Chenopodium amaranticolor are also used.

ELISA (Clark & Adams 1977) is used for leaf roll A,M,S and Y viruses. Absorbance values are read on a 'Multiskan' plate reader (Titertek) linked to a Commodore CBM 800 computer. Data are evaluated and stored using the ELISA data III program (Mitchell 1985). Chloroplast agglutination is used to test for virus X. Confirmatory tests may be done using immunosorbent electron microscopy.

To test for nematode transmitted viruses in field soil (tomato black-ring and tobacco rattle viruses), samples of 1 kg soil (comprising 10 sub-samples from 5-10 cm depth over 50 m^2 field) are baited with two cucumber (cv. Butchers Disease Resisting) and two C. quinoa plants. After 4-5 weeks sap from root samples is inoculated onto five N. tabacum indicator plants.

Viroids

Screening for potato spindle tuber viroid, which does not occur in the UK, is done by tomato/polyacrylamide gel electrophoresis (Harris & Miller-Jones 1981). Duplicate samples are tested using a radioactive complementary DNA probe (Harris et al.1984).

For cultivars in the sub-quarantine facility, additional tests to those shown in Fig. 2 are conducted. Eye-plug plants and all microplants which are grown on to produce tubers are tested.

Bacteria

Ring rot (Corynebacterium sepedonicum), which does not occur in the UK, is tested for by inoculation of tuber heel-end sap extracts into egg plants (Lelliott & Sellar 1976). Microplants received at the sub-quarantine facility are tested using sap expressed from stem bases of glasshouse grown plants. Duplicate samples are examined using immunofluorescent microscopy according to methods adopted by the EEC Ring Rot Experts' Group.

Pectolytic erwinias are tested for by macerating *in vitro* microplants in 5 ml of polygalacturonate enrichment medium (Burr & Schroth 1977) and incubating anaerobically for 96 h at 26°C in Gaspak jars (BBL). A single loopful (2 mm diameter) of the culture medium is then plated onto double layer McConkey pectate medium (Stewart 1962) and after aerobic incubation at 26°C suspect pectolytic colonies are characterised by appropriate biochemical tests (Graham 1972).

CONCLUDING REMARKS

The establishment of pathogen-free stocks by meristem culture (Mellor & Stace-Smith 1977) or clonal selection followed by stem-cutting or micropropagation (Hirst & Hide 1967, Graham & Hardie 1971) with subsequent rapid multiplication by methods utilising stem and leaf bud cuttings (Goodwin 1981) and micropropagation (Goodwin 1980, Hussey & Stacey 1981), and combined with sophisticated disease-indexing procedures (Slack & French, 1983), are now used routinely in many countries for the production of potato nucleus stocks (Foldø 1984, Jordens-Rottger 1984, Mastenbroek & Bakker 1984). Inevitably re-introduction of pathogens will occur during subsequent years of clonal multiplication, but by appropriate hygiene, chemical control and husbandry measures, these risks may be delayed or minimised. Indeed Scottish growers, pioneers in the use of pathogen-free nucleus stocks derived from stem-cuttings, have achieved a marked reduction in blackleg levels in commercial seed potato crops over the last 16 years (Quinn 1985). Reductions in blackleg levels have also been reported from Colorado, USA where stocks have been also derived from stem-cuttings (Knutson 1982). Stem-cutting and tissue culture methods are now recognised internationally as the basis of any scheme to control tuber-borne bacterial diseases such as blackleg (Anon. 1985c).

The main restriction to increasing the production of nucleus stocks and reducing the number of years over which potato stocks must be clonally multiplied is cost. Any further shortening of the production chain must not only reduce disease levels but also increase the value of the crop. For new cultivars or cultivars in short supply, shortening the production chain has undoubted economic benefits, and already 75 000-100 000 microplants are produced annually for this purpose by licenced commercial laboratories from tested parental material supplied by DAFS.

In practice micropropagation has advantages over stem-cuttings: the system is more manageable since most of the initial work is done under controlled environmental conditions, the tuber yield is higher and the cultivar collection can be stored *in vitro* thereby eliminating the need for the annual disease indexing of field grown tubers. However, although high multiplication rates are possible from single tubers, caution should be adopted since occasionally we have detected in field grown microplants undesirable variations in both plant morphology and tuber colour.

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MICROPROPAGATION OF POTATOES FOR VARIETY TRIALS

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INTRODUCTION

The Potato Section of the National Institute of Agricultural Botany (NIAB) carries out agronomic and disease trials on new potato varieties . To avoid the problems of using seed from different sources (Flack 1983) the Institute produces its own seed . Virus testing and initial propagation is carried out at Cambridge, subsequent seed production is at a site in Cumbria within the protected area for seed potato production . In 1983 micropropagation was introduced in order to accelerate seed production so that NIAB Recommended List (RL) trials could begin after only two seasons of seed multiplication .

MATERIALS AND METHODS

Varieties were multiplied by micropropagation to predetermined targets (800 plants for First early varieties, 400 plants for other varieties). Explants were axillary buds from etiolated tuber sprouts . The growth medium was a modified Murashige and Skoog salt mixture (Hussey and Stacey, 1981) . The in vitro cultures were grown at 23°C and a 16 hour day (4000 lux) Culture vessels were 25ml, 60ml and 300ml screw cap glass jars . Final cultures were grown in 9cm sterile plastic petri dishes . In the second week of May the cultures were transported to Cumbria and transferred from the petri dishes to peat pots . The plants were grown under glass for 25 days and then transplanted by hand into the field . Soil around the plants was gradually ridged up by hand and subsequently by machine . The plants were harvested on 31st August . Full details of the method are given in a previous paper (Wooster and Dixon 1984) .

Subsequent seed multiplication followed current seed production practices .

RESULTS AND DISCUSSION

Cultures were initiated in December 1983 and completed by the end of April 1984 . The targets were easily achieved by micropropagation . Losses during in vitro culture due to contamination were less than 5% for all varieties . Losses of plants under glass were significant in the variety Fambo (Table 1). There were losses in the field of transplanted plants in all varieties (Table 1). This was mainly due to grazing by rabbits . Transplanted plants are now protected by electric fencing .

Yields from the micropropagated plants are shown in Table 2; these were higher than expected . Mean tuber number per plant ranged from 6·3 to 19·2 . The harvested tubers were split into two portions . One portion was used to plant a "pre-trial multiplication" in 1984 ie. for RL trials in 1985 . The second portion was used to plant a "main multiplication" for subsequent trials . The weight of tubers planted and harvested in the 1984 pre-trial multiplication are shown in Table 2 .

Eight of the nine varieties entered a series of RL trials in 1985 (Table 2) after two years of seed multiplication . This means that for most varieties RL trials can begin directly after the completion of statutory National List trials (Fig. 1) .

TABLE 1

Number of plants at each stage of the propagation.

Variety	Planted under glass	Transplanted to the field	Harvested
Maris Bard	800	740	613
Ulster Sceptre	800	768	540
Fronika	400	345	269
Fambo	400	99	56
Cromwell	400	376	258
Morag	400	388	318
Santé	400	344	309
Apaché	400	380	256
Jewel	400	360	200

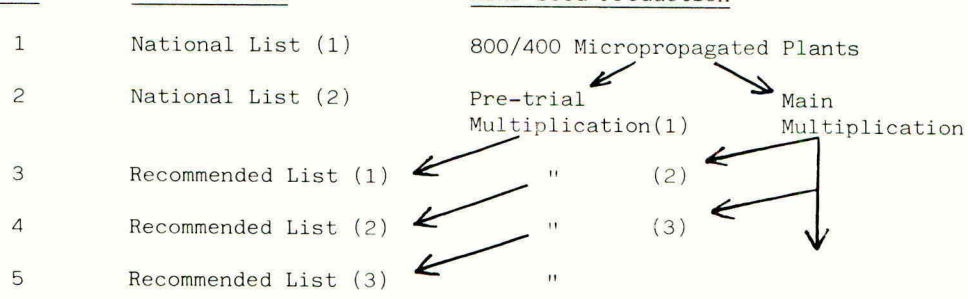
TABLE 2

Summary of seed multiplication 1983 and 1984.

Variety	Micro-propagation 1983	Pre Trial Multiplication 1984		No. RL trials 1985
	Yield*(Kg)	Planted(Kg)	Yield*(Kg)	
Maris Bard	323	115	880	10
Ulster Sceptre	314	115	850	10
Fronika	302	200	1674	7
Fambo	23	-	-	0
Cromwell	155	100	1597	11
Morag	225	46	656	4
Santé	297	125	1488	6
Apaché	219	148	1130	10
Jewel	250	128	1035	6

*Figures are total yield

Figure 1. Seed Multiplication and Trial Programme for RL Candidates



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Poster Abstracts

INTRODUCING HEALTHY PLANTING MATERIAL

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ABSTRACT

Plant material being introduced into the United Kingdom from overseas should be free from pests and diseases to avoid risks to agricultural and horticultural crops already in cultivation and to the environment. The importation of plants and planting material into England and Wales is subject to the requirements of the Import and Export (Plant Health) (Great Britain) Order 1980. Licences may be issued for the importation into England and Wales of plants and plant material of prohibited genera for research and commercial purposes. Pests and diseases that are not established here, or are subject to statutory control, may also require a licence for importation and retention, as determined by the Plant Pests (Great Britain) Order 1980. Applications for licences should be made to M.A.F.F., Plant Health Division, Great Westminster House, Horseferry Road, London SW1P 2AE. Advice on technical matters should be directed to the M.A.F.F., Harpenden Laboratory. Similar arrangements apply to Scotland and Northern Ireland.

NARCISSUS CERTIFICATION IN SCOTLAND

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Narcissus production in Scotland is centred in the north-east coastal area of the country mainly in the Grampian Region. The area has important natural advantages for narcissus bulb production - suitable soil types and a favourable climate. Narcissi are grown on large arable farms which allow a long rotation between crops. In the cool climate virus spread in stocks is extremely slow. The incidence of the important narcissus pests and diseases eg stem and bulb eelworm Ditylenchus dipsaci and basal rot - Fusarium oxysporum f.sp.narcissi is very low.

In response to a request from growers in the area a certification scheme for commercial stocks, based on visual inspection and rogueing, was introduced in 1969. Tolerance levels were set for obvious virus, pests and diseases. This scheme has been successful for several cultivars, and stocks of sufficiently high health standard for export are regularly produced. However, several viruses infect narcissus without causing obvious symptoms or produce symptoms only very late in the season. Furthermore, because of substantial virus infection certain cultivars failed to reach certification standard. Consequently in 1972 a project was started to obtain and propagate virus-free plants of commercially important cultivars, and to introduce these to the industry together with a certification scheme for Virus Tested stocks.

In 1976 the first virus-free stocks were introduced to the industry and the Foundation Stock Scheme was initiated. Commercial propagation is performed in two stages under strictly controlled conditions prescribed by the Department of Agriculture & Fisheries for Scotland. In the first stage which lasts for 3 years twin scaling is used for rapid multiplication and the stocks are grown in vector proof structures in sterilized soil. In the second stage - field propagation - the following requirements are laid down in respect of isolation and site suitability.

Proposed sites must:-

1. have no previous history of narcissus cropping
2. be at least 500 metres from commercial narcissus crops
3. be sampled and tested to ensure relative freedom from virus vector nematodes.
4. be free from detectable soil borne virus
5. be fumigated with dichloropropene

Growing crops are inspected twice during the growing season to ensure freedom from virus infection, pests and diseases.

Stocks of narcissus of Virus Tested origin are eligible for certification at Foundation Stock level for six years after release from protected structures. Thereafter they will be eligible for entry to Elite grade, and this stage has now been reached. The first Foundation Stocks, cvs. Carlton and Sempre Avanti, have been distributed to grower members of the Scottish

Nuclear Stock Association (Flower Bulbs) Ltd and these will be eligible for Elite grade certification in 1986. The isolation and site requirements for Elite grade are as follows.

Proposed sites must:

1. have no previous history of narcissus cropping
2. be 50 metres from commercial narcissus crops
3. be free from detectable soil borne virus

Stocks will be inspected twice during the growing season to ensure freedom from virus infection, pests and diseases.

Five cultivars have now reached Foundation Stock grade and a further five are in first stage propagation. All the commercially important cultivars are included and these stocks will eventually replace existing commercial stocks.

PRODUCTION OF VIRUS TESTED RASPBERRIES IN SCOTLAND

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Virus tested raspberry canes were first made available to growers in the United Kingdom in the late 40's and in the intervening years several changes have been made to the methods of producing high grade stock.

MOTHER STOCK

Foundation stock of raspberry is derived from the mother plants produced and maintained at the Scottish Crops Research Institute. The mother plants are obtained from clonal material that has been tested and found to be free from (a) all known Rubus viruses as assessed by leaf symptoms, graft inoculation to Rubus occidentalis and mechanical inoculation of sap to herbaceous test plants, and (b) "crumbly fruit", a genetical condition in which some druplets abort, the fruit shows uneven development and disintegrates when picked. Mother plants are maintained at all times in aphid proof gauze houses and, to confirm their continued freedom from virus infection, are indexed annually (a) by graft inoculation to Rubus occidentalis and (b) by ELISA to test for raspberry bushy dwarf and black raspberry necrosis viruses. They are also hand-pollinated to identify and eliminate any clones that may have mutated to forms with aberrant fruit set.

FOUNDATION STOCK

Root harvested from the mother plants is supplied annually to the National Seed Development Organisation who sub-contract part of the propagation work to the East of Scotland College of Agriculture. The method of propagation is by root cuttings (Hudson 1954/55) timed to produce a pot grown cane for planting out in selected field sites in November. Propagation and growing on of all the foundation stock is carried out in aphid proof glasshouses and gauze structures to prevent primary infection by viruliferous aphids. Approximately 15,000 pot canes of the main cultivars ordered by the Nuclear Stock Associations are produced each year.

ELITE CERTIFICATE STOCK

In Scotland foundation stock pot canes are planted annually at two separate sites for the production of elite stock. Sites are sampled prior to planting for potato cyst nematode, Longidorus elongatus

an important vector of soil borne viruses, and Pratylenchus penetrans a damaging free living nematode. The isolation requirements for Elite stock are: 1000 metres from commercial fruiting plantations of Rubus; 250 metres from wild raspberries and other wild Rubus species, small areas of domestic fruiting Rubus and stock-cane or standard certificate spawn beds. In practice Elite spawn beds are located well outside of the main fruiting production areas. Cultivars susceptible to Raspberry Leaf Spot Virus are grown separately from cultivars which carry RLSV symptomlessly, eg Malling Jewel. Foundation stock is eligible for Elite grade certification for four years after release, provided unbroken certification is maintained.

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PRODUCTION AND CERTIFICATION OF RED CORE FREE STRAWBERRIES

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The statutory certification schemes for strawberries in the United Kingdom have been outstandingly successful in eliminating virus diseases from strawberry runners but have been less successful in containing the spread of some root pathogens, notably Phytophthora fragariae, the causal organism of Red Core.

In Scotland, the Department of Agriculture and Fisheries for Scotland (DAFS) operates the certification scheme under "The Sale of Strawberry Plant and Blackcurrant Bushes (Scotland) Order 1947". The order prohibits the sale of uncertified strawberry runners in Scotland, and the planting of uncertified runners from England and Wales and Eire. Prior to 1971 the scheme required that two growing season visual inspections were carried out, one in July and another in September but by this time it had become apparent that the probability of detecting red core was remote when strawberries were inspected only in the growing season following planting. In an attempt to improve detection a third examination in the following spring was introduced in 1971. This inspection of runners dramatically increased the detection rate and amply demonstrated the need to find a more reliable method of identifying low levels of infection of the fungus.

In 1978 there was an important breakthrough in the detection of red core on strawberry runners when a root tip bait test for the disease was developed by Dr. J. Duncan at the Scottish Crops Research Institute (SCRI).

This test can detect very low levels of infection, too low to detect by visual inspection. The test was applied on an experimental basis in the 1978/79 season and incorporated fully into the scheme from 1980/81 onwards.

The sensitivity of the root tip test provided conclusive evidence that low levels of infection frequently escaped visual detection and endorsed the view that growers should try to purchase runners which have been subjected to a root tip test. The root tip test is carried out in early winter and results are available before runner lifting commences in the spring. The test is not suitable for autumn lifted runners because soil temperature and moisture may not have reached the optimum level for root infection to occur. At present this is not a problem in Scotland where spring planting is a standard practice, but it is recognised that this will be a problem in parts of the country where autumn planting is preferred.

In Scotland, when it was realised that the root tip test could add a new dimension to the detection of the disease it was decided that foundation stocks guaranteed free from red core should be made available to runner producers. To overcome the risk factor associated with traditional methods of growing strawberries in soil, the foundation stocks are produced by micropropagation. Relatively small numbers are involved at this stage to reduce the risk of introducing aberrant forms. A strict surveillance of mother stocks and progeny is carried out and progeny is kept for several years before the daughter runners are sold for fruiting plantations.

PRODUCTION OF VIRUS-FREE GARLIC BY MERISTEM-TIP CULTURE

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ABSTRACT

All cultivars of garlic (Allium sativum) used for commercial crop production in the UK were found to be infected with one or more viruses. Using electron microscope serology (ISEM) 'decoration' tests, onion yellow dwarf (OYDV) and leek yellow stripe (LYSV) viruses belonging to the Potyvirus group, were frequently identified in most cultivars tested. Shallot latent virus (SLV), a member of the Carlavirus group, was found in one cultivar. In addition to these three viruses, unidentified virus particles were observed in many cultivars that were typical of the Poty- and Carlavirus group. These particles were not 'decorated' by OYDV, LYSV or SLV antisera.

In collaboration with Efford EHS, infected bulbs of the cultivars Blanc de Drome, Fructidor, Rose du Var, Blanc du Connun, Printanor, Moulinin, Ail du Nord and a cultivar originating in the Isles of Scilly have been subjected to meristem-tip culture. Various media were tested for the regeneration of meristem-tips into plantlets, and the most satisfactory medium was found to be Gamborg's B5 containing nicotinic acid (2 mg/l), pyridoxine HCl (2 mg/l), inositol (200 mg/l), sucrose (30 g/l) and agar (0.6%). The optimal levels of kinetin and IAA for shoot proliferation and rooting were determined in a factorial design experiment. Rapid rooting was obtained on the basic medium with no auxin or cytokinin added, and maximum shoot proliferation was obtained on a medium containing 2.5-10 mg/l kinetin and 4 mg/l IAA. Rooted plantlets could be readily established in peat 'Jiffy' pots and hardened-off in plastic-covered seed propagation trays. These plantlets were later transplanted into larger pots containing John Innes No. 3 compost, before being transplanted into field soil in an insect-proofed gauze-house. Plantlets which were transplanted into the unheated gauze-house in October, had developed normal bulbs when harvested the following August.

All tissue cultured plants were indexed for virus by ISEM before transplanting into the gauze-house (6-9 months after tube culture) and during early summer growth in the gauze-house. These tests showed that a high proportion of meristem-tip cultured plants of the eight cultivars were virus-free.