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This report covers the work undertaken for the Apple and Pear Research Council during the period April 1991 to March 1994 at Wye College, University of London. Two versions are presented; the first is a general account highlighting the results of the experiments made and placing the conclusions in the context of earlier assumptions on the life-history of apple canker. The second version is a more detailed account of the methods and techniques used in each experiment, presents the data in full and discusses these results in relation to the scientific literature.

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APPLE CANKER: AN INVESTIGATION INTO THE ORIGIN OF INFECTION IN ENGLISH ORCHARDS

PART 1: A GENERAL SUMMARY OF THE WORK

1. Introduction

Canker, a disease caused by the fungus, *Nectria galligena* is present in all parts of the world where apples and pears are grown but is particularly prevalent in Europe, and is sometimes called European Canker to distinguish it from other somewhat similar disorders in North America. The disease causes serious economic loss to commercial fruit production in two ways; the development of lesions, or cankers in the branches of the wood which may lead to the death of limbs or whole trees and by the development of fruit rots at or after harvest.

The disease has been the subject of research for more than 100 years and a survey of the extensive literature that has been accumulated over this period would suggest that enough is known about the life cycle of the pathogen and the methods of infection to ensure that it can be satisfactorily controlled. However, it continues to be a serious problem in many orchards and is a major cause of rotting in stored fruit, or at least it would be if the only effective control, post harvest dips in the fungicide benomyl, were banned. Indeed, the starting point for the investigation described in this report was the concern for the increased losses that would occur if this ban happened. The only way to effectively control canker rots in this event would be to eliminate all sources of infection in the orchard, an extremely difficult goal to achieve, particularly in very susceptible cultivars such as Spartan, and even Cox's Orange Pippin once the disease becomes even moderately severe. Such experience confirms the maxim that prevention is better than cure, but this can only be achieved if we know where and how the infection originates.

With most fungal diseases of plants the means of dispersal of the pathogen is some kind of spore, and the agent of dispersal is either air or water, with man and other animals providing

an alternative. *Nectria* produces two kinds of spore, asexually formed conidia which occur as cream coloured masses on the surface of new lesions on wood and fruit and the later formed sexual spores, which are produced in flask shaped structures on older cankers. These are red in colour and are about the same size as the egg of the red spider mite with which they can be confused. This can be resolved with a simple hand lens as the fruiting body of the fungus, called a perithicium, has a pore at the top. In moist weather the sexual spores, ascospores, are violently discharged through this pore in groups of eight. The little package of spores within a small drop of fluid can be shot out for up to 2cms, which is thought to enable it to escape from the boundary layer of air and thus be dispersed by wind. However, it is a rather large and heavy spore group relative to the spores of other apple pathogens such as mildew and scab and it must be doubted whether it can be carried as freely by wind currents as those other disease causing agents. Moreover, in very wet weather the rate of discharge from a fruit body becomes so frequent that the pore clogs up and subsequent dispersal can only take place by rain splash and run off, which can carry away the accumulated ooze of ascospores. Similarly, the asexual conidia are formed in dense masses which can only be washed off by rain. These observations would tend to suggest that tree to tree spread of infection can not be as easily achieved by this fungus as it can for scab and mildew.

The received wisdom is that infection occurs through newly exposed leaf scars and wounds such as those caused by pruning. Experimentally it is easy to demonstrate that drops of water containing spores are drawn into the broken water vessels of newly exposed leaf scars and cankers develop at these sites. As the scars become older the self healing process plugs up the broken ends of the water conducting vessels and spores placed on scars 48 hours after leaf

fall rarely result in the development of a canker. Thus each leaf presents a fairly narrow window of opportunity for infection, but it is assumed that as leaf fall extends over many days or even weeks in autumn the period of overall susceptibility is fairly long. A further opportunity occurs in spring with the fall of the scales that cover the buds. Researchers in different countries have concluded that the most important periods of infection, spring or autumn, depends on which has the most favourable weather in terms of rainfall. However, all agree that pruning wounds present a special hazard and recommend the coldest but driest months of mid-winter for this operation.

The control measures which have been developed over the years have targeted these periods of susceptibility, spring and autumn, and have sought to provide a protective coating of fungicide for these presumed sites of infection. To ensure that the fungicide layer is present on the most vulnerable sites most workers have recommended two autumn applications, at least and one in spring. The autumn sprays are usually made at the beginning of leaf fall and when 50% of the leaves are off, which inevitably relies on some redistribution of fungicide to protect scars which become exposed subsequent to application. In the 1930s and 40s the fungicides of choice were based on heavy metals such as copper and mercury which had the additional perceived advantage of accelerating leaf fall and thus reducing the period of susceptibility. Modern organic fungicides, such as dithianon, do not have this advantage even though they have been shown to provide good protection.

Many commercial farms have adopted these spray regimes and on the basis that many of these remain clean, growers remain confident of their efficacy. However, where canker levels are moderate to severe the usual experience is that protectant programmes of this type are

ineffective and then recourse is made to the practice of cutting out wood bearing cankers and painting the supposedly clean wood beneath with the same fungicide containing paints which are used to protect pruning wounds.

It is not always easy to see why some orchards, particularly young ones, become so severely infected so quickly. One possibility is that inoculum originates in hedgerows and windbreaks and it is known that *Nectria* has a wider host range than apples and pears. Poplar is particularly susceptible and with hawthorn beech or ash could provide the focus for infection in those localities where the Grey Alder has not been adopted as the single species windbreak. Alder, which is not susceptible does not provide protection from canker however, as many recent outbreaks have shown.

It is customary to seek an explanation for severe canker in the soil, and the literature abounds in anecdotal evidence that wet heavy soils and high nitrogen levels are conducive to the disease. What is not clear is how these conditions can influence primary infection. Indeed when either of these factors has been tested experimentally no difference in susceptibility to applied inoculum could be detected. However, given the frequency of reports that, for example, the application of high ratios of animal manure to orchards results in severe canker, the phenomenon can not be discounted, even though it cannot be explained in terms of the assumed method of infection.

There are no objective experimental procedures for the assessment of the susceptibility of the cultivars used in commercial orchards, and again the information available is based on the anecdotal evidence of growers. Thus artificial inoculation made by placing a drop of water

containing spores on a newly exposed leaf scar will result in the formation of a canker on every cultivar. In practice, however, cultivars such as Bramley's Seedling are much less frequently severely cankered in the field than say Spartan, which has an unenviable reputation for this disease. Interestingly, some growers have Spartan orchards which are quite healthy, but it is not at all clear how they have succeeded where others have failed. On the basis of available evidence the only hypothesis that can explain such differences is that *Nectria* is not dispersed very far from an infected tree and that with a clean Spartan orchard there was no souce of infection within the critical range.

The work described in this report was designed to address some of these anomalies and had as its primary objective the identification of the source of infection in commercial orchards, with a view to achieving control of both wood canker and fruit rotting without recourse to fungicidal programmes.

It was decided from the outset to concentrate on relatively young orchards in which it might be easier to discover the source of infection than in old orchards and to determine what, if any, contribution infection in the nursery might make to subsequent disease levels. Dr. Margery Bennet, working at East Malling in the sixties had concluded that the nursery was the main source of infection, but this had not been tested nor was there any explanation of how there could be a delay between infection in the nursery and canker appearance in the orchard two or three years later.

2. Orchard observations

At the outset of these investigations several orchards were inspected in detail in Kent, Sussex

and Hampshire. In general these were chosen because of their history of canker and as such represented the most severe aspects of the disease in England. A number of general conclusions were drawn from these inspections:

The most severely damaged young orchards seen were of the recently introduced cultivars, Fiesta, Gala and Discovery. Most of these were on the rootstock M9 which has thereby acquired a reputation for increasing the susceptibility of scions to the disease, although there is no experimental proof for such an assertion.

Most of the orchards visited were provided with windbreaks of Grey Alder, which invariably appeared healthy. Inspection of hedgerows of other species, which included hawthorn and poplar failed to reveal any infection with *Nectria* and it was concluded that in none of the cases examined was the presence of alternative hosts responsible for disease in the orchard.

Likewise, proximity to older infected orchards did not seem to play any part in the distribution of disease in the orchards. Gradients of infection across an orchard were never seen and therefore there was no evidence of spread from areas of high to low infection as might be expected with this splash dispersed pathogen. However, there were many striking examples of individual trees within an orchard being very severely affected whilst their nearest neighbours remained healthy. In most of these it seemed unlikely that this was the result of a single primary infection which spread within the tree to give large numbers of secondary cankers. An analysis of the age of the infected wood suggested, that there were large numbers of infections appearing simultaneously.

There was little opportunity to investigate the effect of soil type, water relations or nutrient status of the orchards visited. However, from discussions with the growers it was clear that certain localities within their holdings were more likely to give difficulties with canker than others. On inspection of such sites, during the summer months there was no obvious explanation for this experience.

One factor which was encountered several times was the provenance of the trees which were infected. For example, in one orchard of the cv. Gala, four rows of trees were very severely cankered whilst adjacent rows were quite healthy. On enquiry it was learned that this block had been obtained from a different nursery from the others and that approximately one quarter of the trees had already been replaced. There were no local sources of infection within the orchard to explain this outbreak, which thus tended to implicate the nursery as the source, whilst the presence of healthy adjacent trees discounted tree to tree spread within the orchard.

Careful attention was then given to the provenance of the trees examined, particularly Fiesta. It was possible to trace a few examples of orchards planted in various localities that had not only come from the same nursery but, in a few instances, from the same batch of production. There was no consistent pattern of infection however, and the severity of canker found varied widely. Interestingly the earliest plantings of Fiesta were not as severely infected as those made more recently, and which have given the cultivar a bad reputation for the disease.

Inspection of nurseries indicated that the frequency of infection of mother trees and stool beds was low. Canker was found on mother trees of Bramley's Seedling and Gala, but the practice

of cutting out cankered wood and indeed the system of pruning used seemed to keep the apparent levels of infection down. One of the nurseries implicated in the production of infected Fiesta was examined in great detail but it was impossible to find a single example of canker. The inference from these observations is that inoculum in nurseries through the liberation of conidia or ascospores from active cankers is almost discountable.

3. A comparison of the isolates of *Nectria* obtained from different localities in England.

During the course of the visits to these orchards samples of cankered wood were collected and returned to the laboratory in order to isolate the pathogen. The original objective of this exercise was to determine if severe outbreaks were related to a particular strain of the fungus, but preliminary experiments which compared their ability to rot apples gave no indication that this was the case. However, it was noted that conidia from some isolates were smaller than others, and this led to a detailed investigation into the morphology of conidia, to determine if spore shape could aid the identification of the source of infection.

Conidia of *Nectria galligena* are cylindrical, with rounded ends and contain up to six separate cells, separated by cross walls called septa. Conidia from a single isolate thus vary in length depending on the frequency of septa. Using a computer aided image analysis system 100 conidia of each isolate were measured for length breadth and septation. The data was then subjected to discriminant and cluster analysis in order to determine the relatedness of each isolate.

Using this technique it was possible to identify three distinct groups or clusters amongst 28

isolates examined. Inspection of the individuals within each cluster revealed that they contained members that had been isolated in several localities and from several varieties of apples, implying that the basis for their relatedness was not either of these two factors. However, the majority of the isolates within each cluster had been obtained from trees which had originated in the same nursery. The isolates from one particular nursery were especially distinctive, being much smaller, on average, from those originating elsewhere.

This technique was found to have a number of limitations and the groupings had areas of overlap that might have been due to changes in conidial size that occurred with age of culture. It was therefore decided to examine the isolates in a less ambiguous way, using techniques comparable to genetic finger printing. The genetic code of all living organisms is based on DNA, found in all cells. By using enzymes which break up the chains of DNA and then separating the fragments into bands by electrophoresis it is possible to detect variation and similarity between isolates. This work was carried out by colleagues at the Queens University of Belfast, Drs. Mills, Brown and Sreenapreasad.

The application of these techniques, best known as RFLP analysis (Random Fragment Length Polymorphisms) again showed that the isolates could be divided into groups. The English isolates belonged to one of four main groups and again the majority of the members of each group could be linked to the nursery which had produced the trees.

The fact that not all the isolates from trees produced in a given nursery belonged to the same group when analysed either by spore shape or RFLP was puzzling if, as was now indicated, the infection originated during propagation. One explanation for these discrepancies might be

the result of exchange of young trees between nurseries to fulfill orders, a common commercial practice. A particular set of isolates from Fiesta which were assumed to be from the same nursery were found to have RFLP patterns characteristic for that nursery and second nursery. On checking his records the nurseryman found that he had bought in trees from the second nursery in order to obtain a sufficient number for dispatch to the customers.

In addition to the English isolates RFLP analysis was made on an isolate from New Zealand, and, interestingly this was found to be quite distinctive from all the others.

4. Symptomless infection of apple trees by *Nectria galligena*

If, as the work described in Section 3 implied, trees become infected in some way during propagation in the nursery then it is necessary to explain how symptom expression can be delayed for one or more years, given that cankers usually become visible within a month or so following artificial inoculation of leaf scars. Indeed, it is necessary to explain how infection takes place in the nursery in view of the apparent absence of actively sporing cankers in most of them. A significant step towards this explanation was obtained by the discovery that wood which had no external symptoms could contain the fungus.

During the course of collecting isolates from orchards whole trees were returned to the laboratory to determine the distribution of infections. Whilst these were being cut up into sections it was observed that areas of shoots remote from the site of an obvious lesion contained brown and discoloured streaks within one or more of the annual rings. These became particularly pronounced within the area closest to the lesion, and suggested that infection could be at least partially systemic. To test this samples of wood were cut from

various locations on trees with one or more cankers. The samples were carefully surface sterilised to remove all trace of external contamination and placed on media which enabled fungi present inside the wood to grow out. Wood of all ages was found to contain a number of fungi, some of which are known pathogens. A significant number of wood samples taken from apparently healthy areas yielded *Nectria galligena*. In general it was found most frequently in wood that was younger than the cankered area and especially in wood that had the stained streaks. It was especially interesting to note that it could be found in the youngest shoots of the current season, even when it was separated by growth of two previous seasons from the zone of an active canker.

It might also be significant that the recovery of *Nectria galligena* from wood was increased if the sample was frozen for a few days. This suggests that freezing increases the competitive ability of the pathogen in relation to the other fungi present. Particularly cold winters are known to enhance canker development.

Such systemic infection was found in a number of cultivars examined and it was concluded that if a tree showed any sign of canker, there was a high probablity that all parts of the tree would contain the pathogen. This would explain how some trees in an orchard become disproportionately cankered relative to their neighbours.

Systemic infection in a young Gala plantation at Wye College was closely followed over a period of 18 months. Shoots of the current year's growth were collected at intervals from June onwards. The first positive isolations were made in July but the frequency of recovery did not peak until late August/September, after which they declined. Whilst these trees had

had an early history of canker on the main stem, cutting out and painting had apparently brought the disease under control. The presence of the fungus in the current year's shoots showed that this treatment merely suppressed the symptoms. A later episode of canker in these younger shoots (see Section 6) was evidently brought about by this systemic infection.

The presence of staining within the wood suggests that the fungus is capable of rising with the sap stream. This was tested by cutting extension shoots at various times during the growing season and placing the cut ends in a suspension of conidia. After 10 days parts of the shoot were examined to determine how far the fungus had penetrated. In shoots cut in July the extent of penetration was relatively small, but in August/September it was found that the fungus had moved up to 10cm. This was consistent with the observations made with natural infections. When the shoots were cut below the junctions with the previous year's wood, penetration was restricted to the junction until August/September, suggesting that there is an anatomical block to penetration before this date. No penetration of the wood occurred in shoots collected after leaf fall or in shoots which were artificially defoliated, which confirmed that transpiration was necessary to enable upward movement of the pathogen.

Sections of wood remote from active cankers revealed the presence of hyphae within the xylem in areas which were stained brown. Using an immunofluorescence stain, based on a monoclonal antibody to *Nectria* (see Section 5) this was shown to be the pathogen. These preliminary investigations suggested that the fungus was discontinuously distributed within the xylem. Thus some vessels contained extensive aggregations of hyphae whilst adjoining vessels contained no trace. Unfortunately the immunofluorescent stain did not react with conidia and it was impossible to detect these, even if they had been present. The question of

how the fungus becomes systemic is therefore unresolved. However, if *Nectria* behaves in an analagous way with other pathogens that inhabit xylem, as in *Verticillium* wilt of hops, it may well produce conidia which get carried up with the transpiration stream. This would explain the discontinuous nature of the colonisation of apple.

An experiment was made to compare the rate of uptake of conidia by shoots of three cultivars, Gala, Discovery and Bramley's Seedling. In the latter there was very little upward movement, whilst in the other two the fungus was found 15cm above the cut after 10 days. Moreover in these two cultivars this method of inoculation induced marked chlorosis of the leaves, beginning with the lowest and progressing upwards to the youngest. Very little chlorosis was seen in Bramley's Seedling and this is consistent with the greater field resistance of this cultivar relative to the others. This might make a useful test to screen new cultivars for resistance.

5. Improved methods for the detection of *Nectria* in apple wood

The discovery that *Nectria* can be found in apparently healthy apple wood was made using the classical technique of placing pieces of surface sterilised wood on media and waiting for the fungus to grow out. This is particularly time consuming as it was found impossible to develop media that were selective for *Nectria*, with the consequence that each colony that grew from the wood had to be sub-cultured before a positive identification could be made.

Following consultation with members of the APRC advisory panel it was decided to divert from the original project plan in order to produce a more efficient method of detection. these are described in detail in Part II, and only a brief summary presented here.

Two methods were assessed. The first was based on the work undertaken at Queens University of Belfast to type *Nectria* isolates using RFLP analysis. Using the ability to amplify sections of DNA through the process of polymerase chain reaction and with the help of primers specific to the DNA of *Nectria* it was found to be possible to detect minute quantities of the fungus in wood.

The second method developed was based on a technique usually associated with the detection of viruses, namely the use of specific antigens for *Nectria* in a Enzyme Linked Immunoabsorbant Assay (ELISA). Monoclonal antibodies were raised by immunising mice with hyphal fragments and the hybridoma cell lines selected were specific for *Nectria*. This work was carried out by Dr. F. Dewey at the University of Oxford. In practice shavings of wood are extracted for *Nectria* antigens and, if present then bind to the McAb which can be detected by a second antibody linked to an enzyme which gives a colour reaction.

Both methods are now available for application in any screening procedure set up to test trees, rootstocks or Mother trees for systemic infection. The ELISA system was selected for the experimental programme conducted up to April 1994 because it was relatively cheap and less demanding of the instrumentation then available. Further refinements of both methods are being explored which could lead to the development of test kits which do not require sophisticated laboratories or skilled technicians.

An additional future use of both methods could be the prediction of rotting before fruit are placed in store.

6. The effect of benomyl on the development of cankers and on systemic infection by *Nectria galligena*

The discovery of systemic infection in apple by *Nectria* has two main implications for commercial production. The first of these is that symptomless infection can be carried through the propagation process in the nursery. The second is that trees in commercial orchards which have had canker are probably systemically infected and the problem is likely to continue unless it can be eradicated. Fungicide programmes designed to protect leaf scars in autumn and spring are unlikely to influence systemic infection.

To eliminate systemic infection a systemic fungicide is required. Benomyl (Benlate) is known to have systemic properties and this was tested in 1992 in the Wye College orchard which contained systemically infected Gala. Benlate, at standared rate, was applied by hand lance to plots of trees at 7 day intervals between June and July. Control plots received no treatment.

The results were assessed in three ways. Firstly, fruit samples were picked from all plots and placed in cold stores until they had fully ripened. Rotting was mainly due to *Sclerotinia* (brown rot) and *Nectria* rots were very low in all samples. Benlate had no effect on *Nectria* rots.

During the winter of 1992/3 the trees in these plots developed die-back of the tips of the youngest shoots and by the spring it was obvious that this was canker and thus constituted a resurgence of the disease that was so damaging in the first years of the plantation. Given that fruit rotting was very low it seems unlikely that this represented fresh infection and was

probably brought about by systemic infection. Benlate treatment reduced this tip die-back by 40%.

A third assessment, made in September 1993 took advantage of the availability of the ELISA test. Shoots of the current season, thus the ones which developed in the year after treatment, were tested for systemic but symptomless infection. Almost all of the trees were found to be systemically infected, and Benlate had no effect on this.

Thus Benlate suppressed symptoms but not infection, which was disappointing. It is not clear whether this was due to a failure of the active ingredient to penetrate into the transpiration stream or whether more applications, perhaps over several seasons would have enabled high enough concentrations to reach the sites of infection. Further experiments are needed, including use of other materials to develop eradicant programmes for existing orchards.

7. Comparison of canker development in trees from different nurseries

The linkage identified between isolates of *Nectria* obtained from orchards with the nurseries that produced the trees, coupled with the finding that trees can be systemically infected without producing symptoms clearly implies that this is a significant route for infection. Confirmation was sought in two experiments that will take a number of seasons to complete. An interim account of these is presented here.

The first experiment was based on a simple assumption; if canker in an orchard planted with trees from several nurseries was randomly distributed then the inoculum source would be local to the orchard. If, on the other hand, canker was more frequent in trees from particular

nurseries, then infection occurred before planting. Trees were willingly donated from 10 nurseries, 5 in G.B. and 5 in mainland Europe. Mr. P. Vinson made an area of land available at Aylesham, Kent where he was planting Queen Cox on M9, which thus became the cultivar for the experiment. To date canker has remained a very minor problem at this site, but has appeared in a small number of trees, produced by only two nurseries. Observatins will continue for a further two years, and ELISA tests will be made in 1994, which should detect any systemic infection.

More convincing evidence that the nursery was the source of much infection was obtained from a second experiment in which rootstocks from three nurseries were budded with the scion cultivar Spartan from a single source. The M9 rootstocks were grown in compost in pots, arranged in a fully randomised block. Bud sticks of Spartan, selected because of the reported susceptibility, were taken from branches which were canker free, although infections were evident on the trees. ELISA tests on these shoots showed that aproximately 30% were systemically infected. Even before budding however, canker was showing up on rootstocks from one of the nurseries.

One half of the rootstocks were budded from budsticks that had been immersed in Benlate for one hour prior to cutting, the other with untreated buds. Cankers began to appear at the graft site in the following winter, and by the time the rootstocks were cut back in the following spring, 40 of the 600 buds had failed from this cause. Analysis of this data revealed that Benlate had significantly reduced this loss but that additionally losses were higher with some sources of rootstock than others.

The rootstock tops that were removed at this time were additionally subjected to an ELISA test. This showed a very significant bias to nurseries, with one showing five times as much systemic infection as another, the heavily infected nursery being the same one in which cankers appeared even before budding.

The experiment will be transferred to the field in the winter of 1994/5, so that observations on canker development can be confirmed. On the basis of results obtained so far there seems every likelihood that systemic, but cryptic infection has been established in some trees.

Benlate clearly suppresses symptom development but what is not obvious at this stage is whether the symptoms in question were derived from infection in the rootstock or scion. Given that leaves are routinely removed from bud sticks, preventing transpiration and thus translocation, the effect of benomyl on the buds must have been largely superficial even after immersion for one hour. However, it is possible that active ingredient was transferred to the graft interface, which could have affected either partner.

8. Conclusions

These investigations have shown by various means that infection in the nursery makes a significant contribution to the amount of canker found in orchards, particularly in the early years of a plantation. The work has also shown that trees with cankers are probably systemically infected, with the consequence that the traditional view of how the disease spreads within a tree needs to be revised. These findings have implications for both growers and nurserymen, which should lead to some changes in practices.

Growers are often reluctant to grub young trees with cankers, and expend considerable resources in cutting out and painting infected areas. This is unlikely to be cost effective and cannot eliminate infection, so that even if a tree seems to "grow away" from the problem it will remain systemically infected. Grubbing young trees with canker seems the best method of control.

It is not known how long a tree can remain symptomlessly infected, nor if cankers which appear on older branches arise from systemic infection or through secondary infection. The complete removal of an infected branch to as low a position as possible, would seem to be the best policy. Scraping cankers back to apparently clean wood is unlikely to suffice.

The use of systemic fungicides appears to control symptoms but not infection, at least if they are used for relatively short periods. Given the risks associated with developing tolerance in apple rotting pathogens to these useful post harvest fungicides coupled with their known impact on the environment, their use can not be recommended. The protectant programmes used in spring and autumn will have little or no effect on systemic infections, but it would be prudent to continue to employ them where the risk of spread from adjacent orchards seems to be high.

There is no information available which can pin point how infection takes place within the nursery. If, as was found with silver leaf of plum and cherry, systemic and symptomless infection can be transmitted by both rootstock and scion wood, then it will be essential to grub out all mother trees and stool beds in which infection has been seen. This is already part of the certification scheme, but should be pursued with more rigour, particularly in stool beds

where canker will not be seen until after harvest. Therefore rootstocks should be labelled to indicate clearly which bed they came from.

In the middle to longer term the diagnostic techniques which have been developed will be very useful in selecting healthy mother trees and stool beds and will be applied first to the second release of the EMLA scheme. This will take some years to take effect. In the meantime the future of the apple industry requires that planting continues.

Growers contemplating planting will be anxious to plant healthy trees from uninfected sources, but inevitably this investigation provides no guidance in this respect. The problem seems to be present in both English and Continental nurseries and it can be anticipated that it will vary between clones and cultivars and probably between years of production. Happily the frequency of extreme difficulty with canker is relatively low and the risks are no greater as a result of these findings than they were before, and should not be used at this stage to guide purchasing or planting decisions. The active collaboration already received from the nursery industry should ensure that the problem is resolved as quickly as possible.