New Horizons in Dutch Elm Disease Control

by Professor Clive Brasier

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Dutch elm disease (DED) first appeared in north-west Europe around 1910, and much of the seminal work on its causes was carried out between 1919 and 1934 by several outstanding Dutch women scientists[1]. UK Forestry Commission research on the disease began in the late 1920s when Dr Tom Peace began monitoring its rapid spread into Britain from its first recorded sites on the continent. By the 1940s this first epidemic had died down after causing losses of 10-40% of elms in different European countries (Figure 1). Indeed Peace, in a thorough review[2], was able to write in 1960 "unless it completely changes its present trend of behaviour it will never bring about the disaster once considered imminent". Such a change did come, however, in the late 1960s with the beginning of a second and far more destructive outbreak (Figure 1). As a consequence, a new era of Forestry Commission (FC) research on the disease began.

This research showed that the new outbreak of DED was caused by an entirely different, far more aggressive DED fungus than that responsible for the epidemic of the 1920s-40s[3], and that the new fungus had been imported into Britain on infested elm logs[4]. What followed was the catastrophic epidemic once feared by Peace. Within a decade about 20 million elms out of an estimated UK elm population of 30 million were dead. By the 1990s the number was probably well over 25 million. Studies on the new DED fungus showed that it differed from the original fungus in almost all its important biological properties. The two pathogens were later described as separate species, *Ophiostoma ulmi* (Figure 2a) being the original and *O. novo-ulmi* (Figure 2b) the new highly aggressive pathogen[5].

A variety of short-term control measures were initiated. At the same time, a wide-ranging research programme was undertaken by Pathology Branch on the processes involved in the transmission of the disease by its bark beetle vectors, the interaction between the two DED fungi, and the spread of *O. novo-ulmi* across the northern hemisphere in order to trace the disease's geographical origins and to provide a framework of knowledge upon which realistic longer term control strategies could be based. Research progress in several key areas will be outlined in this article. First, however, the present disease situation in Britain will be summarised. A general description of the disease cycle has recently been produced[6].

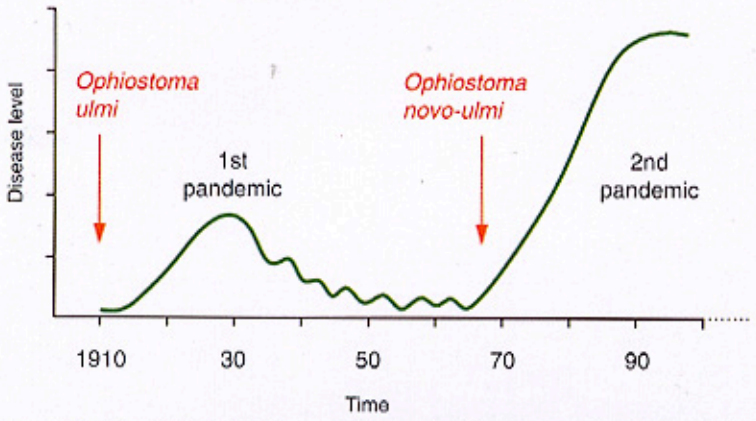


Figure 1. Approximate chronology and impact of the two pandemics of Dutch elm disease in Britain and north-west Europe. Note the decline of the first pandemic in the 1940s.

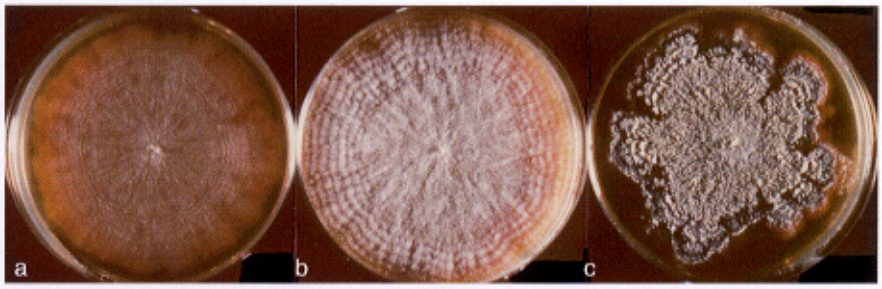
The changing disease status in Britain 1970-1995

Central and southern Britain

Our main native elms, English elm *(U. procera)*, smooth-leaved elm *(U. carpinifolia* or *U. minor)* and wych elm *(U. glabra)*[7] are all susceptible to *O. novo-ulmi*. In lowland central and southern Britain, with predominantly English elm, the new epidemic took rapid hold during the early to mid-1970s[8], leading to the death of most mature English elm by the early 1980s (Figure 3, C-F). There were scattered escapes. Even pockets of mature elm survived occasionally, as in Brighton and Hove where the geographic situation has facilitated an effective and continuing sanitation control programme. However, once most suitable breeding material (inner elm bark) had been used by the beetles the disease virtually disappeared from many south and south-western areas, such as the Chichester-Southampton area, the Gloucester-Berkeley Vale area and the Berkshire-Surrey-Kent area, in the 1980s. During this period suckers growing from surviving roots of English elm and some smooth-leaved elm types appeared in

enormous numbers, together with occasional young seedlings of wych elm. Many small hedgerow elms that escaped the disease have been allowed to mature, in some cases through careful husbandry but often through absence of hedgerow maintenance. Consequently there now exists a numerically massive and increasing elm resource, mainly of small to semi-mature *U. procera*, across much of southern Britain (Figure 3, G1). From Essex to the Welsh borders they probably number many tens of millions.

Figure 2. Characteristic colonies of the three Dutch elm disease fungi: (a) *Ophiostoma ulmi*, cause of the first pandemic; (b) *O. novo-ulmi*, cause of the current pandemic; (c) the recently discovered Himalayan Dutch elm disease pathogen, *O. himal-ulmi*.



In 1982 FC studies on the biology of *O. novo-ulmi*, on disease transmission and on the recent spread of the disease across eastern Europe (Romania to Poland) were combined to produce a prognosis for the future of the disease and of the elm[9]. This prognosis is summarised in Figure 3. As well as predicting the regeneration of the young elms, it suggested that the disease would not die down as had the first epidemic caused by *O. ulmi*, but instead, that the new DED pathogen *O. novo-ulmi* would return, in a continuing cycle, to attack the following generation of small elms once they were large enough to support beetle breeding (Figure 3, F-H). This is what is now happening in southern Britain. In 20 elm plots established by the FC across the south of England, only about 1% of regenerating elms were killed annually between 1980 and 1990, but disease reappeared on a significant scale after 1991[10]. Around the Research Station in the Farnham-Guildford area, no trace of the disease was found during 1981-1987, two separate infections were seen near Godalming in 1988, and by 1990 new infections were scattered across the whole area. By 1994-95 substantial tracts of sucker regrowth and hedgerow elms 3-12 m in height were dead or dying. The above pattern has occurred across most of the old 1970s *U. procera* disease outbreak areas. Some locations, including many parts of the Midlands, are at an earlier stage in the process (<10% diseased). Others, for example the Guildford to Heathrow area, are already quite advanced, with *c.* 50-90% of elms dead or dying in some disease pockets. Indeed the current disease situation is often remarkably reminiscent of the mid-1970s, except that the affected elms are much smaller (Figure 4). We are now approximately at points G1-G2 in Figure 3. A span of about 20 years separates this second wave of disease from the initial outbreak.

Three points should be noted. First, that the regenerating sucker elms are just as susceptible to *O. novo-ulmi* as were the parent trees from which they have developed. Second, that the sudden resurgence of disease in the 1990s probably coincides with the return of the larger elm bark beetle, *Scolytus scolytus*, to the affected areas following its disappearance in the intervening period when little suitable breeding material was available.

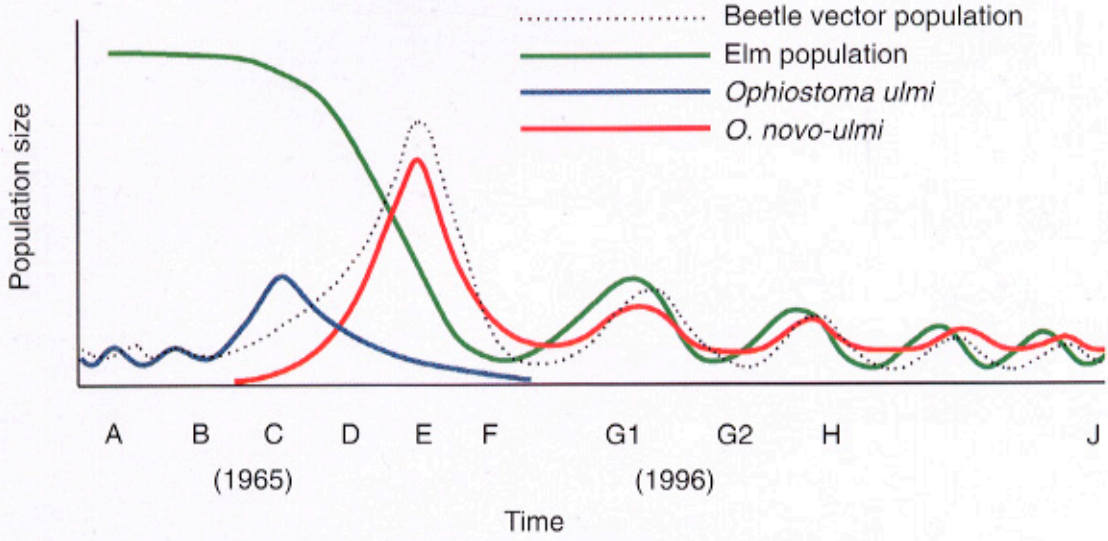


Figure 3. Progress of the current Dutch elm disease epidemic in terms of the elm bark-beetle, *O. ulmi*, and *O. novo-ulmi* populations. Modified from reference 9. See text and reference 9 for details.

Figure 4. Multiple fresh Dutch elm disease infections in a 5 m tall hedgerow of English elm near Odiham, Hampshire, July 1996. *(41576)*

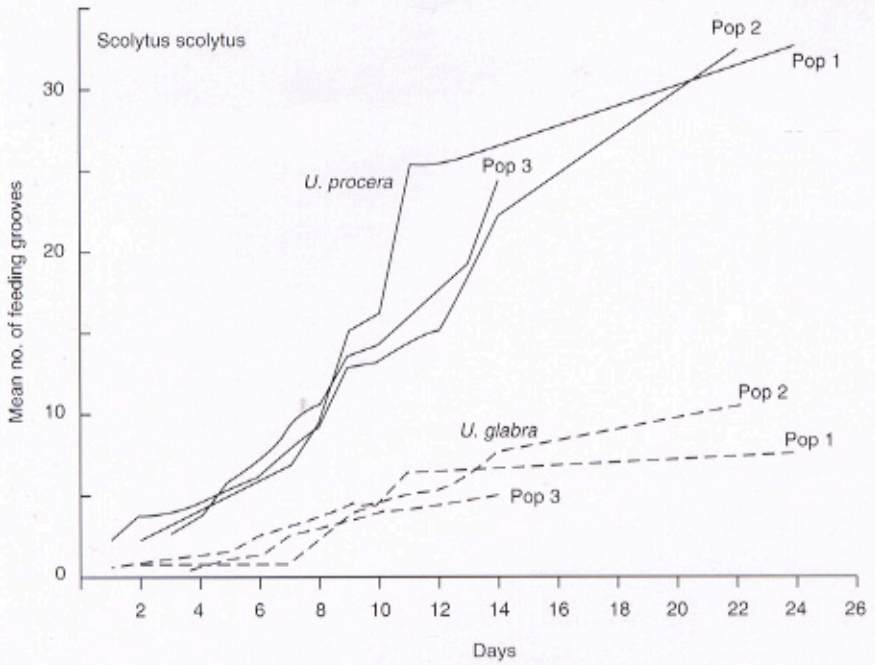


Figure 5. The preference of the larger elm bark beetle, *Scolytus scolytus*, for feeding on English elm *(U. procera)* rather than on wych elm *(U. glabra)*. Three populations of *S. scolytus* were tested. One was a *U. glabra* associated population from Scotland (Pop 3); another a *U. carpinifolia* associated population from Manchester (Pop 2); and the third a *U. procera* associated population from Devon (Pop 1). J. F. Webber, previously unpublished. See also reference 12.

S. scolytus probably migrated back from neighbouring parts of Britain where it has survived. The smaller beetle, *S. multistriatus*, may actually be the first to return to an area, since it can use smaller diameter branches as its breeding material. However FC research shows that *S. multistriatus* is a very ineffective vector of the disease, in contrast to *S. scolytus*[11]. Third, the best way to conserve hedgerow elms at present may be to keep them trimmed, since prominent elms are more likely to attract the bark beetles for feeding.

If a majority of the regenerating elms die over a 10-15 year period, then losses in central and southern Britain may number a million or more trees annually. Certainly DED remains by far our most destructive tree disease. However, although further cycles of disease can be expected (Figure 3, H-J), the elm will survive to provide a contribution to future landscapes. It remains an enormous potential resource.

Cornwall and East Anglia

In the early 1970s the rate of disease progress was markedly slower in the smooth-leaved elm populations of East Anglia and the Cornish elm *(U. carpinifolia* var. *cornubiensis)* populations of the south-west peninsula[8]. The majority of mature Cornish elm and East Anglian smooth-leaved elms have now been killed by the disease. However smooth-leaved elm is highly variable[7] and even now certain local East Anglian smooth-leaved elm clones have suffered only limited losses, with some isolated trees or significant groups of mature trees surviving. Many examples are in woodlands or on woodland edges. Some of these clones are being propagated by local authorities as possible sources of resistant material for replanting. They do not necessarily posess a higher level of resistance to the Dutch elm disease fungus. Many factors can lead to reasonable 'field performance', and these clones could prove highly susceptible if inoculated. All smooth-leaved elm varieties are believed to be introduced into Britain from central and southern Europe[7] and some, being beyond their natural climatic range or site conditions, may be growing rather slowly and producing smaller springwood vessels

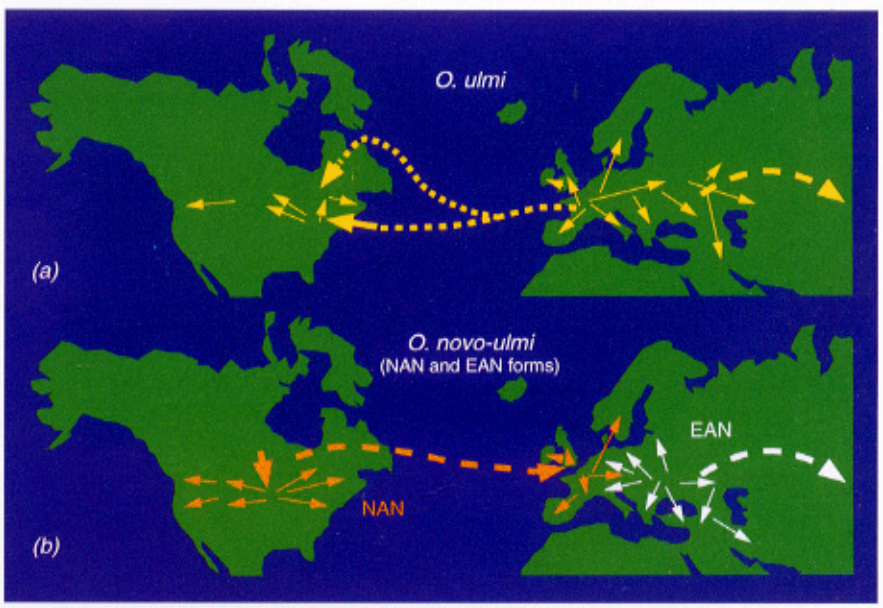
restrictive to the fungus. Good field performance may also involve resistance to beetle feeding or breeding, or involve a natural biological control of the fungus or beetle. Some smooth-leaved elm types have very pendulous twigs when mature, a feature which could make them unattractive to the beetles for feeding.

Scotland and north-west England

Epidemic progress has also been much slower on the predominantly wych elm *(U. glabra)* populations of Scotland and north-west England[8]. The result is that the first wave of the 1970s epidemic is still active and continuing in these areas today. At least three likely causes of this slower progression of disease are apparent. First, *U. glabra* does not sucker like *U. procera* or *U. carpinifolia*[8], hence it suffers less from disease transmission via root grafts[8]. Second, although *U. glabra* is considered even more susceptible to *O. novo-ulmi* than is *U. procera*, it is much less favoured by the bark beetles for feeding[12] (Figure 5). Third, a competitor of the elm bark beetles, the fungus *Phomopsis,* is a common, rapid invader of the bark of newly dying wych elm, thereby acting as a competitor of the elm bark beetles which normally breed in the bark.[13] *Phomopsis* appears to exert a strong natural biological control of the beetle populations of the north and west[13]. Fourth, climatic constraints probably reduce the disease activity of the pathogen by producing fewer opportunities for beetle-originated infections in the summer. The climate may also restrict the size and number of annual bark beetle generations as compared with southern Britain or continental Europe[14]. Such factors have aided a successful disease containment campaign within the Edinburgh city limits.

Nonetheless, the disease is very active in Scotland. It has moved into *U. glabra* populations that were not affected by the first DED epidemic, such as those in the Glasgow area. It is continuing to push northwards, particularly on the east coast north of Aberdeen. This northwards expansion probably reflects the fact that *O. novo-ulmi* has a lower optimum temperature for growth than did *O. ulmi*[7], and the much greater epidemic momentum that *O. novo-ulmi* has generated, allowing *Scolytus scolytus* to expand beyond its previous northern territorial limits.

Figure 6. The spread of (a) *O. ulmi* and (b) *O. novo-ulmi* during the first and second pandemics of Dutch elm disease. Composed from Forestry Commission sample surveys of the pathogen across the Northern Hemisphere in the 1970s-1980s *(O. novo-ulmi)* and from international publications and FC surveys *(O. ulmi)*. Solid arrows show natural migration from original centres of appearance. Dashed arrows indicate subsequent spread via importation events. Note: (i) *O. novo-ulmi* exists as two distinct forms, equivalent to subspecies, called the North American (NAN) and Eurasian (EAN) races. They have different geographical distributions and their centres of original appearance and subsequent spread are shown here in orange (NAN) and white (EAN) respectively. (ii) The evolutionary and geographical origins of *O. ulmi* and of the two races of *O. novo-ulmi* remain unknown. Adapted from reference 16.



New approaches to the problem

We now have a situation across both Europe and North America where the host (the elm) and pathogen *(O. novo-ulmi)* are seriously out of balance: the pathogen is too aggressive for its host (Figure 3, C-G). This is partly because *O. novo-ulmi* is an introduced pathogen that has not co-evolved with native European or North American elms. In addition, some natural enemies of the fungus or of its beetle vectors may be missing from the system.

Because of this host-pathogen imbalance, traditional 'front line' disease controls such those offered by quarantine, sanitation or chemical control[8,15] have been largely overwhelmed by the sheer momentum of the epidemic or undermined by human fallibility. What is needed are radical new approaches to the problem, based on better scientific knowledge, which individually or collectively, can help restore the system to balance more quickly than nature can achieve by itself.

In discussing longer term control prospects, it is important to appreciate that Dutch elm disease, like HIV, or tuberculosis, is an international problem[16] (Figure 6) that requires an international perspective embracing the whole epidemic area - currently from North America to the western Chinese borders and south to New Zealand. Changes in pathogen

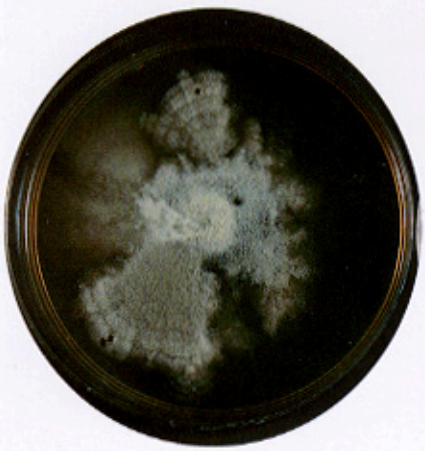
behaviour occurring in Kansas or Uzbekistan today could be occurring on our doorstep tomorrow.

Main options for control include (i) reducing the effectiveness of the beetle vector population; (ii) increasing the resistance of the elm population; and (iii) lowering the aggressiveness of the pathogen. Regarding the beetle vectors, much work has been done on investigating the transmission of the disease[11] and in characterising beetle pheromones as lures for use in sanitation monitoring[14], but no new avenues for control have opened up. Recent research by the Forestry Commission has therefore concentrated on the fungus and on the elm, with special emphasis on the following.

Origin of the disease: discovery of a Himalayan link

Dutch elm disease was unknown before 1900, yet we have had two pandemics in this century each caused by a unique Dutch elm disease fungus[5,16] (Figure 1). Together, they are responsible for one of the most catastrophic environmental events this century, yet their origin remains a mystery.

Figure 7. A d-infected culture of *O. novo-ulmi*, showing the typical irregular unstable growth pattern associated with d-infection.



It is important to establish their origin (1) to understand why such explosive tree pandemics occur and whether they are likely to occur on other trees, and (2) because to identify the geographical centre of origin (or 'endemism') of the disease may present new opportunities for its biological control.

A research programme on the disease's origin was begun in 1979. Detailed surveys of the DED pathogens were carried out across Iran, parts of the former Soviet Union, central and eastern Europe and North America[16]. These produced a picture of the spread of *O. ulmi* in the first pandemic and of *O. novo-ulmi* in the current pandemic (Figure 6). China, which has often been held to be the centre of origin of DED because of its many disease resistant elm species, was surveyed in 1986. No evidence of the disease was found, though native elm bark beetles were common. It was concluded that China was most probably not the geographical origin of the disease. Two alternative hypotheses were presented. First, that *O. ulmi*, *O. novo-ulmi* or both had appeared recently via an unusual evolutionary event through human influence. Second, that they had an origin in the Himalayas, a major floristic region not surveyed for the disease[16].

In 1993 a survey was conducted in the Kullu and Sutlej valley areas, Western Indian Himalayas[17]. This led to the discovery of a third Dutch elm disease fungus, associated with beetle breeding galleries of Himalayan elm bark beetles on the local *Ulmus wallichiana*.

Typical beetle feeding wounds were also seen on the elms. The fungus has been characterised and named *O. himal-ulmi*[17] (Figure 2c). Significantly, *O. himal-ulmi* has proved to be just as pathogenic to elms as *O. novo-ulmi*, and to produce comparatively high levels of the wilt toxin, cerato-ulmin.

While it has not solved the question of the origins of *O. ulmi* or *O. novo-ulmi* directly, this discovery has opened up new approaches to research. First, it has raised the possibility that *O. himal-ulmi* was at some time introduced into Europe or North America, and gave rise to *O. novo-ulmi* by hybridising with *O. ulmi*[17]. This possibility is now under investigation. Second, no wilt disease was seen on elms in the Himalayas. The disease therefore appears to be relatively quiescent and endemic to the region. There is thus an opportunity to understand the natural balance between the elm, fungus and beetle vector in an endemic rather than an epidemic disease system. It may offer important new opportunities for biological control: there may be natural competitors and predators of the fungus and beetle populations in the Himalayas that are missing from the DED system in Europe and North America and could be exploited for biological control purposes[17]. Much may therefore be gained from investigating the ecology and population dynamics of the Himalayan system.

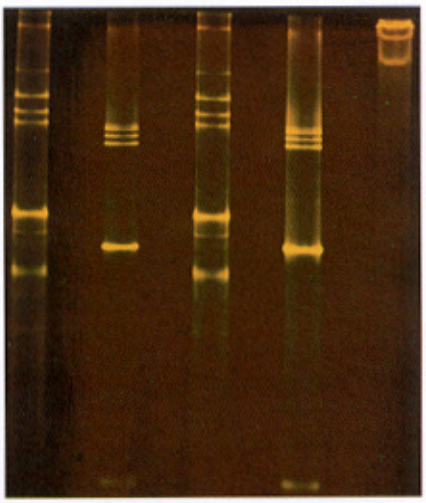
A wider issue raised by the discovery of *O. himal-ulmi* is that such a highly aggressive tree pathogen can exist unknown and unidentified in a major forest region of the world until now. This has considerable implications for our approach to quarantine and forest protection. In particular, it raises a question about the number of exotic pathogen threats not so far identified.

D-factor 'mycoviruses' as potential biocontrol agents

One approach to restoring the balance between the elm and the pathogen is to reduce the pathogen's aggressiveness. In 1983, studies on the fungus' 'tissue incompatibility system' led to the discovery that it had a disease of its own, now known as the 'd-factor'[18]. D-factors undoubtedly have the potential to reduce the aggressiveness of *O. novo-ulmi*.

D-factors are naturally occurring virus-like agents, located in the fungus' cytoplasm, which spread from one

Figure 8. How d-factors are visualised in the laboratory. The multiple dsRNA (double stranded RNA) segments of d-factors revealed on a polyacrilamide gel plate. The segments range from approximately 0.3 to 3.0 kilobases of dsRNA in size. *(34764)*



fungal isolate to another via hyphal fusions. They can severely debilitate *O. novo-ulmi*, inducing slow, ragged 'amoeboid' growth (Figure 7) and reduced sporulation[19]. Many different d-factors have now been identified. Most are associated with double stranded RNA segments (Figure 8), which probably encode for them[20].

Field studies using artificial beetle feeding wounds (Figure 9) show that d-factors can increase the number of *O. novo-ulmi* spores required for infection of English elm beyond the spore loads carried by most vector beetles[21]. Thus the d^2-factor (d-factors have been numbered d^1-d^n) increases the number of spores required for infection from *c.*1000 (healthy isolate) to *c.*50 000 (d^2-infected isolate). D-factors therefore have the potential to break the cycle of Dutch elm disease at the beetle feeding stage[22]. In addition, population studies on *O. ulmi* in Europe and North America suggest d-factors may have been instrumental in the unexpected decline of the first DED epidemic in Europe in the 1940s (Figure 1). Similar studies show that in the present epidemic, d-factors markedly influence the behaviour of *O. novo-ulmi* at epidemic fronts, and are involved in a remarkable and rapid change in its population structure from being clonal to being highly genetically variable[23].

D-factors are the fourth component of the DED system: it is an elm-fungus-fungal virus-beetle vector system. D-factors not only exert a natural biological control of the fungus[19,22], but may have considerable potential as artificial biocontrol agents. Consequently, they are being studied with a view to their possible release, either unmodified or genetically manipulated, for biocontrol purposes. Studies on physiological differences, molecular profiles and population structure of d-factors are underway with the support of the Pilkington Trust. These are aimed at characterising weak, moderate and strong d-factors[24,25], assessing which d-factors might be most appropriate for release and at determining how different d-factors might interact with each other if they were released. Molecular studies are also in progress with Imperial College and the Gatsby Trust on the detailed genetic structure of d-factors and on the way that they influence *O. novo-ulmi* at the cellular level[26], again with a view to manipulating them to greater ecological effectiveness. One objective is to insert the d-factor into the fungal nucleus, as has recently been achieved for a similar virus of the chestnut blight fungus. This could make a d-factor behave like a nuclear gene, and allow it to be spread more effectively among different genotypes of *O. novo-ulmi* in nature.

Recently, three places have been identified at which there is just a single clone of *O. novo-ulmi* free from d-factors. One is at the new disease outbreak in Auckland, New Zealand; one in Washington DC and the third in Oregon, USA[27]. These populations could be very suitable for an experimental release of d-factors to assess further their biocontrol potential. They are also ideal study sites because only the smaller, less effective beetle vector, *S. multistriatus*, is present. D-infected fungus of the same genotype as the local fungal clone could be released on captured beetles. If these beetles fly to join the local beetle population, the d-factor could be introduced into the fungal population. Either a single d-factor or a mixture of d-factors could be used[25].

It is hoped that an experimental release may soon be initiated. It should be emphasised that research on d-factors as biocontrol agents is relatively new, long-term and carries no guarantee of success. However d-factor influence is one of the most likely ways in which the aggressiveness of *O. novo-ulmi* will become attenuated in nature, with or without human intervention.

Figure 9. A typical beetle feeding wound in the crotch of a healthy elm twig. *(41336)*

Genetic engineering of elms for resistance

Another way of restoring the balance between elm and pathogen would be to increase the baseline resistance of European and North American elms. One difficulty with traditional elm breeding is that it usually involves incorporating resistance from exotic Asiatic elms such as the Siberian elm, *U. pumila*, which are not suited to the UK climate and have a rather different arboreal form[15,28]. Resulting hybrid material may therefore be unsuitable in terms of their susceptibility to mild winters or late frosts, their susceptibility to other pathogens such as *Nectria*, or simply their shape. This is not to say that elm breeders will not, one day, produce a resistant elm ideal for UK requirements.

English elm, *U. procera* (Figure 10), is greatly prized in southern Britain for its 40 m height, straight bole and dense globular 'figure-of-eight' crown as well as for its timber. It is well suited to the UK climate, although probably imported from Iberia[7]. An ideal replacement for English elm would therefore be, quite simply, a disease resistant English elm (or, as appropriate, a resistant wych elm, smooth-leaved elm or Cornish elm).

Figure 10. Mature English elm, *U. procera*, showing its characteristic irregular outline. English elm grows to c.40 m tall. *(34764)*

In 1992 a research programme was initiated between Horticulture Research International, the Forestry Commission and Abertay University to investigate the potential for genetic manipulation of English elm. Good progress has been made to date. Forestry Commission *U. procera* clone SR4 was brought into tissue culture from leaf petioles. Nutrient and hormone conditions were then manipulated to regenerate shoots and roots from the callus tissue[29] (Figure 11). Protocols for transfer of novel DNA into living elm cells were then developed by adapting the widely applied method of using the gall bacterium, *Agrobacterium tumefasciens*. As a result, marker genes causing pigmented tissue have now been successfully and stably inserted into elm plantlets, i.e. genetic transformation of English elm has been achieved[30]. At the same time, a range of unique anti-fungal proteins supplied by Zeneca UK have been assayed for their activity against *O. novo-ulmi* in culture. A pilot anti-fungal gene may be inserted experimentally into *U. procera* shortly. Anti-beetle genes may also be considered for insertion. The activity of these genes will initially be assayed in tissue culture and regenerated plantlets and later assayed in young woody plants.

English elm is so far proving an excellent model system for genetic manipulation of broadleaved trees. Other elm species such as *U. carpinifolia*, *U. glabra* and *U. americana* are also under investigation. If the insertion of resistance genes proves promising during the initial trials, the material may be submitted for licensing according to current environmental regulations, with a view to testing it

under field conditions. Obvious environmental concerns to be considered are the risk of 'escape' of novel DNA beyond the engineered elm plants, and the likelihood of the disruption of 'friendly' or non-target fungal or insect populations.

It is thought that any resistant English elm would be used mainly for specialised landscaping or urban requirements. Exotic hybrid elms or genetically engineered disease resistant elms are probably not the solution to problem of the abundant 'wild,' regenerating sucker or seedling elms already in the field. For these elms, it would seem more appropriate to aim for a better ecological balance between the disease and the host.

Wider implications of Dutch elm disease research

The immediate reasons for seeking to control Dutch elm disease are fairly evident. Returning the elm to its traditional place in the landscape has both high potential amenity and economic value and high historical and cultural significance. Indeed, cultural association with the elm in Britain probably dates back at least to the bronze age, when many favoured elm varieties including the English elm were probably introduced[7]. Dutch elm disease is also introduced into Britain by man, via the modern timber trade. There is therefore some responsibility to restore the loss.

The DED epidemic itself has greatly raised public awareness of trees and tree issues. It has also had a considerable influence on the general practice and philosophy of forest pathology. For example, it has underlined the dangers of introducing 'exotic' pathogens. There is clearly a risk of further 'DEDs' in the future. Learning how to control destructive epidemics following imports of exotic pathogens is part of the current learning curve of forestry practice. So too is learning to predict how such introduced pathogens may adapt to their new environment, and learning to develop and apply knowledge-based control measures within a highly complex ecological system.

Indeed, the DED experience has also underlined the value of approaching forest pathogens through an understanding of their ecology and population biology. In this context, the discrimination of *O. ulmi* from *O. novo-ulmi* on behavioural characters has demonstrated, along with similar studies, that the traditional fungal taxonomy based mainly on morphological characters provides inadequate quarantine protection for our forests: biologically distinct organisms have all too often been classified as the same species just because they are morphologically similar[31]. Population studies on the DED fungi have also raised the question of rapid evolution of forest pathogens[32]. FC research shows that *O. novo-ulmi* continues to change at a remarkable rate.[32] The resulting awareness has led to the suggestion that the newly discovered 'alder *Phytophthora*' could well be a product of recent rapid evolution[33].

Beyond these issues, the DED research programme continues to open up new avenues for control. The discovery of the 'd-factor' is an obvious example. Indeed, virus-like agents such as the d-factor may have considerable potential for control of many forest pathogens. Beetle feeding preference studies have established a new character for use in elm breeding: resistance to the beetle. Studies on transmission of DED have highlighted the beetle feeding groove as a weak point in the disease cycle and a the target area for bological control by d-factors. The discovery of the endemic form of DED in the Himalayas may present further opportunities for biological control.

Finally, it should be emphasised that other possible approaches to the control of DED continue to be explored. Indeed, much recent FC research is not covered in this review. It ranges from pilot studies on induceable resistance in elm[34], to studies on pathogenic behaviour of non-toxin producing isolates of *O. novo-ulmi*[35], to studies on the molecular evolution of the three DED pathogens[36]. With limited resources it is necessary to set clear research priorities and to concentrate the current effort on those areas considered most likely to achieve success.

Figure 11. Young plantlets of English elm, *U. procera*, regenerated from callus tissue. *(Horticulture Research International; 910306)*

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