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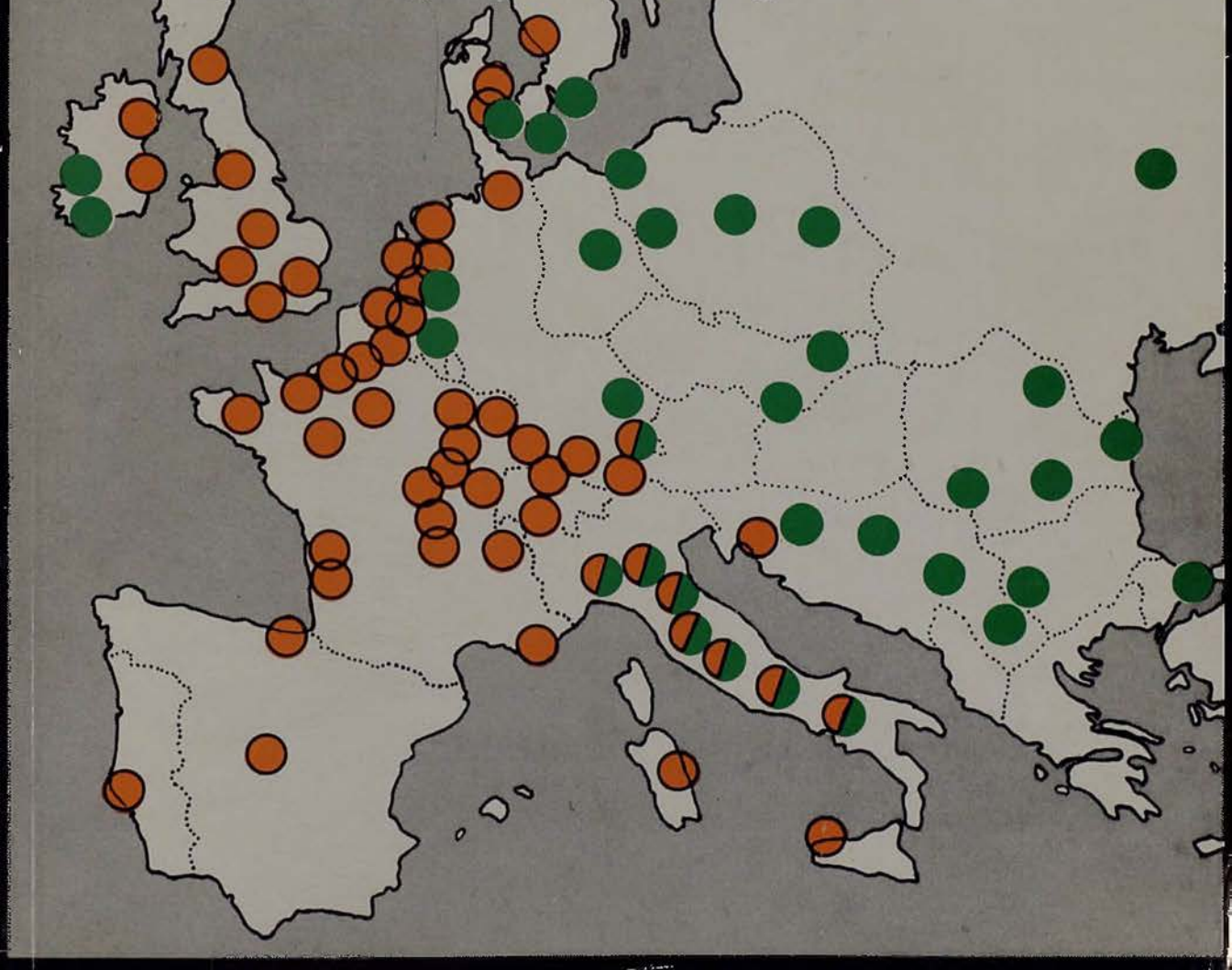
Research on Dutch elm disease in Europe

Edited by D A Burdekin



Cover

Summary of the known positions of the Eurasian (EAN) and North American (NAN) races of the aggressive strain of *Ceratocystis ulmi* in Europe in 1981. Green, EAN race; red, NAN race. Based on identifications of the fungus in artificial culture from isolations made from over one thousand samples collected by Dr. C. M. Brasier (see pp. 96–104). The distribution of the non-aggressive strain of *C. ulmi* is not shown.

FORESTRY COMMISSION BULLETIN
No. 60

Research on Dutch elm disease in Europe

PROCEEDINGS OF THE EUROPEAN ECONOMIC COMMUNITY RESEARCH SEMINAR, GUERNSEY, CHANNEL ISLANDS, 30th MARCH — 1st APRIL 1982

Edited by D. A. BURDEKIN, B.A., Dip. Ag. Sci.,
Chief Research Officer, Forestry Commission,
Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH, U.K.

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Introduction

D. A. BURDEKIN

Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH, U.K.

Dutch elm disease is one of the most important tree diseases that has been found in the northern hemisphere. It was first recognised in 1919 in Picardy in France and during the following two decades it became epidemic throughout Europe and North America. It was called 'Dutch' elm disease as a tribute to the intensive research programme undertaken by Dutch scientists at that time. It was soon established that the disease was caused by a fungus, *Ceratocystis ulmi*, and was transmitted from diseased to healthy elms by the elm bark beetles *Scolytus scolytus* and *Scolytus multistriatus*. It is perhaps interesting to note that there is still some argument about the 'correct' scientific name for the fungus and the Dutch prefer to use the name *Ophiostoma ulmi*. This is not the place to argue the pros and cons of fungal nomenclature, merely to note that scientists cannot always agree!

The epidemic of Dutch elm disease in the first half of this century was very damaging to the elms of Europe and North America but was much less dramatic than the devastating epidemic which occurred in Europe during the 1970s. An upsurge of the disease started in the late 1960s in Britain and soon spread to many western European countries. A very active research programme was initiated in Britain about this time followed by a resurgence of activity in Holland. Following a classical series of studies in Britain it was established that a different strain of the fungus, termed the aggressive strain, had been introduced into Europe from N. America, probably in the late 1960s. The aggressive strain was considerably more damaging to European elms than the non-aggressive strain which was probably responsible for the earlier epidemic.

By the mid 1970s the aggressive strain of *C. ulmi* had been found in several European countries and a number of scientists in these countries became involved in research on the disease. It became clear that a co-ordinated European programme of research was urgently required and that an ideal opportunity existed for the European Economic Community in Brussels to assist in this goal. Since 1977 the EEC has provided funds for an annual research seminar of scientists involved in research on Dutch elm disease where research results and control methods could be freely discussed. A series of remarkably successful meetings have been held since that time in Britain, Holland, Germany and the Channel Island of Guernsey. The papers in this Bulletin are those presented at the 1982 meeting held in Guernsey and they represent a unique collection of papers on many aspects of this complex problem.

In addition to providing support for the co-ordination of research programmes in Europe, the EEC has also made a very important contribution to the European research programme. Funds were made available for the employment of additional scientists to work on the project. Two were employed in Britain to work on various aspects of the fungus and the insect, one in Holland to work on fungal/host relationships and one in West Germany to study the bark beetle.

The papers which appear in this Bulletin summarise the results of recent research undertaken by scientists from six members of the European Community. It is, of course, impossible to make a comprehensive review of the achievements of the overall research programme in a short introduction. However a few highlights can be mentioned and a number of important conclusions can be reached.

Three main approaches to the control of Dutch elm disease have been investigated during the past decade and these are:

(a) control of the elm bark beetle

(b) control of the fungus

(c) selection and breeding of resistant elms.

The traditional method of controlling the disease has been by sanitation felling, the cutting and destruction of dead or dying elms so that the source of the breeding beetles is eliminated. This method of control has been tried in many countries and has met with varying degrees of success. Some examples of successful and not so successful sanitation felling are presented in the first section of this Bulletin. Where valuable individual trees are at risk techniques

for the injection of chemical fungicides or perhaps biological agents such as bacteria have been developed.

A number of studies of the elm bark beetles in Europe has given a much greater insight into the conditions governing the activity of the beetle. The means by which the beetle is attracted to dead and dying elms is now much better understood. The chemical nature of the attractants (pheromones) produced by *Scolytus scolytus* has been identified. These chemicals have been used in the field to monitor beetle populations where control operations are in progress.

Studies of the fungus have followed two main directions, one towards a better understanding of the effects of the fungus on the host tree and the other involving a detailed study of the variability of the fungus. A clearer picture is now emerging of the mechanisms by which the fungus can invade and kill the vascular tissues of the host.

The studies on the variability of the fungus have considerable implications for the selection and breeding of elms. As a result of recent studies it is clear that at least two forms of the aggressive strain exist, one invading Europe from the west and the other from the east. Until there is a clearer view of where the fungus originated, the possibility of finding further variants must remain open.

Whilst the pathologists and entomologists have been at a peak of research activity during the past decade, the long term selection and breeding programme for resistant elms has continued in Holland. Selected new clones at least moderately resistant to the aggressive strain of *C. ulmi* have been released to the nursery trade. A number of promising elm clones have been planted in a series of trials in most member states of the European Community. New collections of potentially resistant elms have been made in Asia.

What conclusions can be drawn at this stage in the research programme?

In order to safeguard existing elm populations from the ravages of Dutch elm disease a vigorous sanitation felling programme can markedly reduce the impact of the disease. Valuable elms, e.g. in important streets and parks, can be protected from infection by the injection of chemical fungicides and perhaps also of biological agents such as bacteria. A number of factors both climatic and biological can affect the rate of progress of the disease and these factors are now much better understood.

In future plantings of elm, there is an important place for elms resistant to the disease. New clones are being introduced which not only show resistance to Dutch elm disease but are also suited to particular environments (e.g. exposed situations, urban and rural road sides, etc.). At the same time it is vitally important to ensure that we have a full knowledge of the variability of the fungus so that new clones do not fall prey to new fungal strains.

PAPER 1

The Dutch elm disease control campaign in Guernsey, Channel Islands, 1976–1981

G. F. RILEY
Plant Health Officer, States of Guernsey

Importance of the elm to Guernsey

Approximately 80 per cent of the total number of trees on the Island of Guernsey consist of elm trees, estimated to number some 200,000. The elms are varieties of the smooth-leaved elm *Ulmus carpinifolia*. At least two types are present. One variety which is very common on the Island is var *sarniensis*, the Guernsey elm. Although certainly slightly more resistant than common English elm *Ulmus procera*, it is undoubtedly susceptible to Dutch elm disease (DED) and, although disease initiation is slower, the symptoms can spread very rapidly within the tree once it has become infected. The other main type on the Island appears to be a form of the Continental elm *Ulmus carpinifolia*. Total destruction of the elm population in the Island would result in a major change to the Island's general landscape, and a great reduction in the landscape value of many areas.

Tourism is a very important part of the Island's economy, and on ecological and aesthetic grounds, the importance of the elm to Guernsey cannot be over-emphasised. Again, as the most important tree species in the Island, the elms have a great value in sheltering large areas of the Island from strong winds. Horticulture is one of the main industries in the Island economy, and the loss of elms would lead to a great reduction in shelter from the wind generally, and could have a major effect on the cost of heating glasshouses. In 1976 it was estimated that for each mile per hour mean wind-speed increase, the annual cost of such heating would increase by approximately £¼ million. Thus it became essential that control measures against DED should be implemented in an attempt to slow the spread of the disease, and that replanting schemes should be carried out before the majority of the tree cover in the Island was lost.

Brief history of the Dutch elm disease campaign

The Island Government became concerned at the progress of the disease in the United Kingdom in the early 1970s, and at the identification and spread of the disease in Jersey from 1974 onwards. A preliminary amount of surveying and voluntary observation was then undertaken in Guernsey.

In 1976 an Island-wide survey was carried out by the States Committee for Horticulture, with the help of parish constables who reported details of suspect trees. It was unfortunate that severe drought conditions in that year may have masked symptoms of the aggressive strain of the elm disease fungus *Ceratocystis ulmi*; trees suffered from and were weakened by the drought, and Honey fungus *Armillaria mellea* which is widespread in Guernsey, was considered the main agent causing the death of the elms. It is almost certain that the aggressive strain reached the Island in 1976 when such abnormal drought conditions induced premature senescence masking the presence of the aggressive strain which, from the pattern experienced in the United Kingdom and Jersey, almost certainly had a high incidence in late summer. In the United Kingdom the hot weather was very favourable for elm bark beetle breeding and huge populations built up. This phenomenon of beetle breeding in drought-stressed trees certainly occurred in Guernsey. Nevertheless, at this time we had the benefit of experience gained elsewhere, and were able to prepare contingency measures which could be put into effect immediately the disease was identified.

Confirmation of the presence of the aggressive strain was made by the British Forestry Commission in August 1977, and Mr. B. J. W. Greig of the Forestry Commission visited the Island to help in the assessment of the situation, and confirmed that the contingency plans were sensible and should effectively minimise disease spread.

It became clear that to distinguish between the aggressive and non-aggressive strains in the situation which then existed was an unnecessary exercise. Not only would the necessary tests have caused delays, but if trees infected by the non-aggressive strain of *C. ulmi* had been left standing they would have provided ideal breeding grounds for the beetles. The total number of trees felled in this year was 1,136 ; these were considered diseased, or had

beetles breeding in them. The cost of the felling programme was approximately £20,000, equating to an average cost of felling and burning of just under £18 per tree.

The Island Government then had to decide whether a complete control campaign was to be put into operation. We had the benefit of the experience gained elsewhere, particularly in East Sussex and Jersey. It was estimated that left unchecked a substantial proportion of the Guernsey elm population would probably be killed within seven years, and replacement trees would take at least 15 years to have any protective or scenic effect. However a factor in Guernsey's favour is that as an Island it is in almost total isolation, and thus the area involved would generally enable early detection of infection to be made.

The following policy was therefore laid before the Island Parliament:

1. that the ban on the importation of unbarked elm wood be continued under existing legislation;
2. that temporary legislation be introduced to ban the felling or lopping of elm trees without permission, and to direct owners to inform the authorities when elm trees were felled (this was mainly to ensure that no possible source of infection was left on the site);
3. that provision be made in its budget for £50,000 to cover control measures in 1978;
4. that a tree replacement programme be investigated and that the States would meet the full cost of providing prescribed trees used for the replacement of elms destroyed.

This was accepted by the Government. A special co-ordinating sub-committee was set up comprising representatives of the Committee for Horticulture (overall control), the Board of Administration (felling of trees, etc.), and the Island Development Committee (tree replanting), with the overall execution of the campaign under the Plant Health Officer. Thus an effective liaison was established, with a great deal of emphasis being placed on the need for public co-operation in reporting suspect trees; therefore public relations and education became an essential part of the exercise, so that the minimum amount of legislation could be brought into being.

Thus, in 1978 there was a systematic survey to identify diseased trees, followed by sanitary felling. 2,318 diseased trees were identified. Their felling, which was carried out throughout the summer, was completed in January 1979. Felling was undertaken by the States Works Department at a total cost of £46,705, an average of £18.65 per tree. Trees were felled, removed for burning, and stumps debarked for this cost.

Although root transmission is a significant factor in the spread of the disease because so many of the Island elms are in hedgerows and in narrow lanes, effective severance is difficult and expensive, and except where a particularly fine stand of elms is involved, it is not practical.

In addition to the 1978 survey, a comprehensive study was made of the trees in a quarter of a mile band across the Island, indicating the species of tree and soil conditions, together with an aerial survey to provide a photographic study of the contours. The objective was to provide full information to assist with the tree replanting programme. In 1978 over 5,000 saplings were provided free of charge.

The policy accepted by the States for 1979 was that provision of £100,000 should be made to deal with an estimated 4,000 infected trees in that year. The number of infected trees found in 1979 was in fact 1,375. Although it appeared that the control measures were having a positive effect, it was felt that a thorough examination should be carried out to ascertain the precise reason for the reduction in the number of infected trees. Again it was decided to invite Mr. Greig, Chief Forester with the Forestry Commission's Pathology Branch to visit the Island in September 1979 to make a critical assessment.

The disease situation

Although there were notable infection centres, the overall disease level on the Island in 1979 appeared to be low, and the majority of diseased trees discovered that summer were at old infection sites recorded in 1978. Where severe outbreaks were seen, the trees wilted very rapidly, and died quickly once the initial infection had become established, subsequent spread was generally through root transference of the disease. Twig samples were collected from thirteen sites on the Island; of the nineteen isolations, seventeen were typical in growth rate and morphology to the North American strain of *Ceratocystis ulmi*, and two were identical to the non-aggressive form.

Breeding activity by the elm bark beetle appeared to be low in the Island during the summer, and only on one site were trees found in which beetles were attempting to breed in dying trees. Previously (in 1977) beetle breeding had been, in contrast, very extensive in diseased trees and logs. Similar situations were found in East Sussex and Hove in 1978.

The losses in other British control areas were also generally less in 1979:

	1978	1979
Brighton	266	173
Hove	416	298
Edinburgh	326	233
East Sussex	9,083	7,730

The cost of the elm disease control programme in Guernsey for 1979 was £34,000 (£22.87 per diseased tree felled). The policy accepted by the States for 1980 was that provision should again be made for £100,000 based on an estimate of 3,000 infected trees for that year; 2,241 elms were found to be infected and were felled at a cost of £70,000. New varieties of resistant elm, 'Plantyn', 'Lobel', 'Dodoens' and 'Groeneveld' were imported from Holland to be monitored and grown under supervision. Pheromone beetle traps of improved design were set up to enable accurate monitoring of beetle flight and of their population. The policy accepted by the States for 1981 was that provision should be made for £150,000 based on an estimate of 5,000 infected trees for that year; 2,298 trees were felled at a cost of £95,000.

Thus it can be seen that during the five years since the introduction of the elm disease control campaign Guernsey has lost 9,374 elm trees representing approximately 4.7 per cent of the estimated elm population. In keeping losses to such a low level the campaign has achieved a remarkable success. It is thus worthwhile examining the factors involved, and in particular the future lines of development needed in order to keep this control.

Co-operation for control

The sanitation programme remains the basis for control through the felling of diseased trees as soon as possible after infection, in particular those trees in which disease is identified in June and July. Wherever possible, timber from felled elms is immersed in water for a period of twelve months, then salvaged. This treatment is often not practical due to difficulty of sites or size of trees, nevertheless it is an attempt to overcome political objections to burning. There are however many difficulties to be overcome in felling trees on an island which offers many miles of narrow lanes with trees growing on the banks, cliff land (almost inaccessible sometimes), and built-up areas which necessitate the trees being felled in segments prior to removal. These factors contribute significantly to costs.

We have introduced new techniques following the setting up of the EEC project on Dutch elm disease. This project has been invaluable in enabling us to benefit from the expertise to be found within the European Community. We have introduced beetle monitoring and beetle trapping, using traps baited with pheromones. We are using arboricide injection plus pheromone to reduce the beetle population; the experience gained in 1981 will be put to good use in 1982. An appreciation of climatic factors and a knowledge of the biology of the beetle are important for the successful application of this method. While we still consider that it is the overall population of elm that must be protected, we intend to experiment with fungicides on individual trees. We are also interested in evaluating the possible biological control method of using the bacteria *Pseudomonas* spp. We will continue to follow the progress of resistant elms, and maintain a close liaison with the research work being carried on elsewhere.

Between 1978 and 1982, 34,144 forest trees other than elms have been supplied free of charge to the public, and the demand shows no sign of decreasing. Twenty species are involved, and care is taken that no one type predominates. The most popular appear to be silver birch, mountain ash, sweet chestnut, evergreen oak, poplar and willow. Advice is given as to their suitability for the site. A free stake and a bag of fertiliser are provided.

The support from the public of Guernsey in reporting suspect diseased trees has been a fundamental reason for success, bearing in mind the difficult geography of the Island. Significantly this was greatest in 1981, indicating that they just do not want to lose their elm trees. Such co-operation in a closely knit community is a delicate affair indeed. Detailed explanations as to why the campaign is necessary, attending to individual sites where the loss of a tree poses a particular problem, or delaying the felling (in particular where farmers have standing crops) by mutual agreement, and above all notifying the owner of a tree prior to felling, sounds straightforward until one gets involved in boundary disputes following centuries of the Norman law of inheritance!

Adequate legislation is now in force to enable the necessary action to be implemented, but it has not yet been necessary to use it. It is worthy of note that permission to lop or fell an elm must be obtained, and also that when an elm tree falls or dies this must be reported. The object here is to avoid piles of logs left to act as beetle-breeding sites. We are tolerant of non-diseased elm wood being used as fuel during the winter months, but will not have such wood exposed during the summer months.

It is interesting to note that the Island of Sark has also decided to take measures to protect their elms, including the felling of diseased trees during the winter months. In the summer we co-operate

by installing beetle traps and help with disease identification if requested. Thus one of the smallest independent Islands in Europe also benefits from the expertise and research sponsored by the EEC.

The future campaign must now be considered as an attempt to reduce the loss of elms through the measures already indicated, while always being cognisant of research being carried on by larger countries, and by the international co-operation we have already been fortunate enough to have received through the EEC Dutch elm disease project.

Disease control is often unpredictable, and although the island situation offers many advantages, other factors can come to bear. The spring and early summers of the last five years have been notoriously cool; in the context of the elm disease outbreak we have yet to experience a prolonged spell of hot weather in April/May, and the consequent effect on beetle flight. It is reasonable to believe that fresh infection along our south-east coastal strip in 1981 came from Jersey in the late summer of 1980, where the control campaign has been abandoned, and when the weather conditions were optimum for the beetle to fly or be blown across. Such late summer infections are not apparent until the following year.

Thus there can be little ground for complacency, and each year must be assessed on its own merits. However the Island of Guernsey is fortunate in having the political willpower to continue the campaign, with public co-operation to an extraordinary degree, and is fortunate to still have the tree cover so necessary for the Island and which for many years to come only the elm can provide.

PAPER 2

Dutch elm disease control campaign in Jersey, Channel Islands, 1974–1982

G. JOURNEAUX

Manager, Parks, Gardens and Playing Fields, Department of Public Building and Works, Jersey

Introduction

The commonest tree in Jersey is the elm, in particular the Jersey elm (*Ulmus carpinifolia* var *sarniensis*). This was planted in large numbers during the early and middle part of the 19th century, mainly as a windbreak for the numerous orchards in the Island at that time and also as a supplementary cattle fodder. Therefore, the greater proportion of these elms are situated along the hedgerows throughout the Island and form an important part of the Island's scenery. A survey in 1975 estimated that of a total of 500,000 trees, some 40 per cent were elms and thus more than that proportion of trees in the hedgerows were elms. The loss of these elms would have a disastrous effect both on the agriculture of the Island and also on the scenic attraction of the country from the tourist point of view.

Recognition of the elm disease outbreak in Jersey

The aggressive strain of *Ceratocystis ulmi* was first identified in Jersey on the 20th June 1974. In July 1974 it was found in a group of trees half a mile south of Jersey Airport and also in a group of trees adjacent to the north boundary of that airport. A helicopter survey of Jersey at that time showed a tongue of infection reaching two miles east of the airport and also a small infected area in the east of the Island, but separate from the first area.

The States' authorities were notified of the presence of the aggressive strain of *C. ulmi* in Jersey and were advised of the seriousness of the situation with the implications it had for the elm population of Jersey. Although the disease was not identified until June 1974 the extent of its spread at that time indicated that it must have been present in 1973 and the condition of the trees south of the airport in 1974 showed that in all probability these trees were originally infected in 1972.

With the identification of the disease in 1974 steps were taken using the Destructive Insects and Pests (Jersey) Law, 1960, and on the 5th December, 1974, the Dutch Elm Disease (Jersey) Order, 1974, became Law. This Law applied to infected elms only and this restriction in authority was to have a significant effect on the campaign in the following years.

The elm disease control campaign

The sanitation felling of diseased elms began in January 1975. Initially the campaign was run jointly between the Department of Agriculture and Fisheries and the Department of Public Building and Works. The Agricultural Department, using their Plant and Bulb Inspectors, took on the responsibility for surveying and marking infected trees and generally implementing the Law. The Department of Public Building and Works hired contractors to fell and dispose of the trees and supervised their operations. From the start of the campaign all the infected trees were completely destroyed by burning. During the first two years the trees were taken to two sites, one in the west and one in the east of the Island and then burnt. Later more and more of the trees were burnt on or near the place where they were felled until by 1981 only a small volume of wood was burnt at one official site. Initially four contractors were employed to fell the trees, one of these proved unsatisfactory and the number was reduced to three.

In 1976, discussions took place between both Committees and it was agreed that the campaign could be run more efficiently by one Committee and as the legislation was vested in the Committee for Agriculture and Fisheries that Committee took on full responsibility for the campaign forthwith. For the next two years the campaign was run by the Agriculture and Fisheries Department using their staff from the Produce and Bulb Inspection Section. The job of felling the diseased elms was allocated to a variety of tree felling contractors and although this spread the work load it made control of the operation much more difficult. As well as carrying out the survey the inspectors now had to monitor the felling and disposal of the trees; all this in addition to their other duties.

In 1979 at the suggestion of the Committee for Agriculture and Fisheries, the Department of Public

Building and Works took over the complete control of the campaign although the legal authority for it remained with the Committee for Agriculture and Fisheries. Responsibility for the Dutch elm disease control operation was transferred to the Public Works' Department on the 1st May 1979. Mr. P. Perree who had been involved with the sanitation felling campaign as a Produce Inspector was appointed Supervisor, Dutch elm disease. In addition three Field Officers were also employed at the same time. These latter had backgrounds in farming in Jersey and were thus familiar with the local countryside. Thus for the first time since the Dutch elm disease campaign started in Jersey we had personnel working full time on it.

At this stage it was decided that the work should continue throughout the year, that identification and marking of infected trees would be done during the winter as well as in summer and autumn. After two months' initial training the team began work in the field in July 1979. The Island was divided into 14 sections using a 1:5000 map. Two covered very little land area so that effectively there were 12 sections each about 17 sq km. Three contractors were appointed and one Field Officer attached to each. Each contractor was given four sections in which to operate.

Both the surveyors and the contractors started in the west of the Island and moved eastwards following the prevailing wind. In this way it was hoped that the chance of emerging beetles flying against the direction of the felling operation might be reduced. This appeared to be the case as the density of the beetle population was found to increase as the contractors moved east, with a very heavy infestation amongst elms at the extreme eastern edge of Jersey.

When the Field Officers completed their first survey, usually by mid-July, they returned to the west and started a second survey. They spent an hour or so each day with their contractors, during which time they checked that the work was being carried out satisfactorily. Whilst on site they surveyed the immediate vicinity for newly infected trees which were felled there and then. During the winter, in between checking the activities of the contractors, the Field Officers carried out a third survey and also debarked elm stumps where they knew the beetle population had been high.

While carrying out these surveys the Field Officer carried a map on which to plot the location and numbers of infected trees. This information was transferred to a master plan which was copied and given to the contractor. Infected trees identified in the area worked by the contractor were marked in a different colour to those marked on the general surveys. After the section had been completed the number of trees were counted on the map and the contractor paid for that number.

At the beginning of each season (i.e. June) a price per tree was agreed with the contractors. The price was £6.00 per tree in 1975 and £12.40 in 1981. This sum was paid to the contractor for every elm he felled over 6 inches (15 cm) diameter at the base. However, he was obliged to remove every infected tree, even those under 6 inches diameter, and had to burn every part of each tree felled. He was to be responsible for any damage he might cause. The Department replaced any broken fence with a stake and wire fence. Each contractor was required to fell and burn every infected tree which had been marked as such on a 1:5000 grid square map and had to leave the area clean before being paid.

The success of the system can be measured by the fact that only six trees colonised by beetles were found by the first survey of 1980. It had been estimated that some 4,000 trees would succumb to the disease that year through root transference of the fungus. Nevertheless the prospect of a dramatic reduction in the total number of infected trees seemed hopeful. However towards the end of July widespread aerial infection of elms was seen to be taking place. When mapped it was apparent that the infection was much denser in some areas than in others. These areas were checked out and a number of stacks of elm timber were discovered. Although the Law gave us no authority to search property in the course of our surveys, by chance and in some cases through information received, we discovered 40 stacks. Each stack was certified by the States' Entomologist as being colonised by beetles and was burnt. The States' Entomologist gave it as his opinion that an average stack contained a quarter of a million beetles, that in some cases the first brood had already emerged and that the beetles in the stacks were in the process of producing a second brood that season. In addition to cut and stacked logs a number of cases were found where the trees had been felled, the branches removed and the trees left to become hidden by nettles, brambles, etc. The number of diseased elms in Jersey felled from 1975 to 1981 is given in the table below:

Year	Trees felled
1975	7,500
1976	4,500
1977	3,700
1978	6,000
1979	9,500
1980	12,500
1981	14,500

In an attempt to persuade people to co-operate in Dutch elm disease control a publicity campaign was set in motion. A pamphlet was produced showing in simple terms what the disease was, what it did, how it spread and also how to prevent stacked timber being colonised by the bark beetles. These pamphlets were distributed to parish halls, schools, garages, to farmers through the Farmers' Union, even to the Women's Institutes. Over two weeks advertising time was bought on Channel Television and a full page taken up with the local paper. This was done in December 1980, all to no avail. Throughout the winter of 1980/81 more stacks were discovered. The publicity campaign was repeated in June 1981. Reaction to both campaigns was nil.

In December 1980 an amendment to the Law was proposed whereby the Public Works Department would have to be notified within four days if healthy elms were felled so that officers of the Department could check their whereabouts and whether they had been treated in such a way as to prevent their being colonised by the bark beetle. Discussions between the Committee for Agriculture and Fisheries and the Public Works Department and the Law Department went on for twelve months but before any final decision was taken, events intervened.

Because of the apparent success of the sanitation felling in 1979/80 it was estimated that the number of trees to be felled during 1980/81 would be considerably less than in 1979/80 and that the total number including those lost through root transmission of *C. ulmi* would be less than 5,000.

By autumn 1980 it was obvious that this would not be the case, the number rose to 14,500 and the money budgeted for 1981 had to be increased, bringing the total to £150,000. This money was exhausted by November 1981 with some 10,000 trees still to fell. The estimated number of trees to be felled during 1982 rose to 25,000 with an expenditure of £300,000. With an annual infection rate of 25,000 it was calculated that, if this rate was maintained, all the remaining elms would be lost within seven to eight years and without a sanitation felling campaign this period would be four to five years. This meant that over the next eight years, not allowing for increased costs, the expenditure which would be incurred would be in the region of £2½ million with an increase at the most of four years in the life of the elm population of Jersey.

During the first years of the control operation the infection rate was 3.75 per cent, which if maintained would have given a period of approximately thirty years before the elm population disappeared; thirty years during which replacement planting with other species could ameliorate the effect on the landscape caused by the loss of 40 per cent of the Island's tree population. The annual cost of control up to 1978 was in the region of £40,000. The present infection rate (1981) is 20 per cent, with an annual cost in the region of £300,000. The felling and disposal cost per tree rose from £6.00 in 1975 to £12.40 in 1981.

In view of the estimated expenditure of at least £2½ million during the next eight years, and taking into account that the period left for replacement trees has decreased from thirty to eight years the Public Works Committee advised the States of Jersey to abandon sanitation felling as from June 1982. Only those trees identified as being infected before June 1982 would be felled. Thereafter the Public Works Department holds itself responsible only for removing any infected elms bordering roadsides that constitute a hazard. The money saved by discontinuing the sanitation felling will be used to boost a replanting programme, both by supplying trees and advising landowners in the care of trees after planting as well as the care of such parcels of woodland as exist in Jersey.

Methods of utilising elm timber

In view of the opposition from a section of the population to the destruction of elm wood and the understandable feeling that by burning elms we were destroying a valuable resource, various methods of utilising elm timber were considered:

1. *Temporary storage*

To immerse in water for a period of six months. This was a non-starter as, apart from reservoirs, Jersey has no suitable area of water and the Jersey New Waterworks Company Limited were not in favour of using their reservoirs for this purpose.

2. *Production of charcoal*

Although this was a possibility it would appear that there was no ready market either in Jersey or the United Kingdom. In addition charcoal from elm wood is not of a particularly high quality.

3. *Setting up a sawmill*

This appeared to hold out most promise especially as the proposal was that the mill be set up within the Prison grounds and that it would be operated by prisoners. However, although such a mill was a viable proposition to produce planks and firewood while the numbers of elms being felled were high, with the eventual disappearance of the elms the quantity of available timber of other tree species

would be too small to justify the cost of such a mill. This coupled with the fact that the public was already investing some £¼ million annually in the campaign led the Public Works Department to the conclusion that it could not justify the expenditure of £80,000 required to establish the mill.

Other methods of elm disease control

In Jersey we have considered three additional methods of controlling the disease, one by controlling the beetle and two by controlling the fungus.

1. *Cacodylic acid*

This chemical is used to reduce the beetle population by killing elm trees, attracting beetles to them and then inhibiting their ability to breed (see O'Callaghan this volume). This method is an aid to sanitation felling and possibly if it had been available in Jersey in the early years of the disease there it might well have been a useful tool. However, by the time it did become available it was felt that it would have little overall effect where beetles were breeding in their millions in cut timber, a situation over which we had little control.

2. *Ceratotect injection*

In Jersey at the present time there are some 120,000 elms within an area of 50 square miles (130 km²). The injection of Ceratotect is a lengthy process requiring specialised equipment. It is not effective if the proportion of crown infection exceeds 5 per cent. We considered that with 120,000 trees spread over 50 square miles we should be unable to identify a 5 per cent infection and treat it in anything other than a small number of cases. Added to this was the necessity of obtaining a large amount of equipment together with trained operators if we were to treat enough trees to make the expenditure in time and money worthwhile. This method was rejected therefore as a major tool in the fight against Dutch elm disease but it would seem to have a place in treating individual trees of particular value.

3. *Trichoderma viride*

A small quantity of this fungus was sent to Jersey in February 1981 from a firm in Guernsey. It was sent for experimental use to Mr. Bradshaw, Plant Pathologist at the Howard Davis Experimental Farm in Jersey. Contact was made with Dr. Ricard in Sweden whose organisation produces the fungus in pellet form for inoculation.

Trichoderma viride is a biological control agent which is introduced as pellets into holes drilled into the tree. The pellets contain hyphal fragments as well as spores. In addition 50 per cent of the pellet consists of food material plus acid and buffer to inhibit bacterial action which might kill the fungus.

From the information we were given about *Trichoderma* it seemed that it had much to recommend it:

(a) It is said to have no deleterious effect on the tree in which it lives.

(b) It is claimed that it will remain in the tree for up to ten years.

(c) The method of seeding the tree using a ⅜ inch (10 mm) auger at 4 inch (10 cm) intervals along a spiral around the trunk causes a minimum of damage.

(d) A tree of say 18 inches (45 cm) diameter can be treated in 20 minutes.

It was decided therefore to field test elms with *Trichoderma* both as a curative and as a preventative treatment. In March 1981 a number of individual elms known to be infected with *C. ulmi* were treated, and during the summer of 1981 two field trial areas were laid down, one in the west and one in the east of the Island. These two areas are of approximately 210 acres (85 ha) and 120 acres (48 ha), and 90 per cent of all trees within them were seeded with *Trichoderma*. In 1982 we used pellets not only of *Trichoderma* but also of *Scytalidium* which produces the antibiotic scytalidin and which is said to inhibit or destroy *C. ulmi*.

The method of seeding a tree is simple and trees down to 2 inches (5 cm) diameter can be treated. Using a ⅜ inch (10 mm) auger, a hole is bored to the centre of the tree and thereafter, using a battery-operated hand drill, holes are drilled into the cambium at intervals of 4 inches (10 cm) in a spiral around the trunk. The fungal pellets are introduced into the holes using a special applicator invented by the head of our field team. Each hole is sealed with bitumen. Seeding of the trees can only take place while the trees are dormant as the movement of the sap breaks up and ejects the pellets before the hole can be sealed. Therefore seeding must take place between November and March.

The first three elms treated were 40-50 feet (12-15 m) high with diameter at breast height of 18 inches (45 cm). They were marked as being infected in July 1980 and were treated with *Trichoderma* in February 1981. At that time staining of the cambial layer was observed at the base of the trees and sections of the wood were sent to the Forest Research Station at Alice Holt Lodge where the fungus was identified as the aggressive strain of *C. ulmi*. During the spring of 1981 these trees came into leaf and remained so until leaf fall in the autumn. The leaves were of a good colour though noticeably smaller than normal and this plus the absence of

leaves on branches that had died completely, left the trees with a thinner crown than normal. A total of 20 infected trees were treated during February and March 1981. The trees described above were still living a year later

It is in my opinion too early to come to any definite conclusion as to the effectiveness of *Trichoderma* as an agent to combat *C. ulmi.* However, it can be said that trees infected with the disease in 1980 and which in our experience we should have expected to find dead soon after the leaf flush in spring 1981, remained alive throughout the summer of 1981. Whether these trees will continue living during the spring and summer of 1982 remains to be seen, but our initial results with *Trichoderma* gave us cause to be optimistic.

With the abandonment of the sanitation felling in mid-1982 it is expected that the number of infected trees will rise rapidly during the next two years. Therefore the Jersey authorities considered that if possible an alternative elm disease control treatment should be offered to the public and that this should be the use of *Trichoderma.* However it was decided that individuals wishing to take advantage of the treatment for their elm trees should pay for its cost. Two schemes are run side by side. In the first scheme teams from the Public Works Department seed trees at a fixed charge per tree. The second scheme is only open to *bona fide* growers who are sold pellets at cost and instructed in the method of seeding the trees.

Conclusion

From January 1975 until June 1982 the States of Jersey carried out an Island-wide sanitation felling campaign in an attempt to control the spread of Dutch elm disease. From May 1979 when responsibility for the campaign was taken over by the Department of Public Building and Works a highly efficient operation was mounted. This was possible because we were able to look back and learn from the successes and failures of the previous five years, plus having the total commitment to the operation of the Supervisor of the Dutch elm disease campaign and his three Field Officers.

It is my opinion that the failure of the felling campaign has been due to three factors:

1. Our lack of success in convincing the populace and in particular the farming community of the necessity of carrying on a felling campaign.
2. The failure of the authorities at the beginning of the campaign in 1974 to grasp the nettle firmly by the hand and place a complete ban on unlicensed tree felling throughout Jersey.
3. The escalation in the demand for wood as fuel.

During the past two years the market for firewood has increased greatly. The use of chainsaws has made felling trees and converting them into logs so easy compared with the previous method of felling with axe and saw that the rate of tree felling can be increased with less physical effort than previously was the case. In addition farmers have learnt to recognise early infection in their trees and have been felling before our teams have been able to mark them. The production of firewood logs has also contributed to the increase in the beetle population. Logs stored in suburban gardens furnish breeding grounds for the beetle and the chance of finding these logs is remote.

The *Trichoderma* treatment came to our notice at a time when we were beginning to feel doubts as to the success of sanitation felling in Jersey. If we were to have any chance of saving even a small proportion of the elms in Jersey clearly some other method had to be found. We considered that treatment with *Trichoderma* showed the greatest promise and so we chose it. Only time will tell whether we have chosen wisely or not.

Editor's Note. It should be emphasised that treatment of elms with *Trichoderma* has not, at this stage, been investigated in controlled experimental field trials.

PAPER 3

Control of Dutch elm disease in Britain

B. J. W. GREIG and J. N. GIBBS

Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH, U.K.

Introduction

Dutch elm disease (DED) has had a devastating effect on the landscape of southern Britain. The last full Forestry Commission survey in 1978 indicated that 70 per cent of the 23 million elms over 6 m tall had been killed (Gibbs, 1979). By 1981 it was probable that about 20 million trees had died, with only some populations of the East Anglian smooth-leaved elm *Ulmus carpinifolia* and Cornish elm *U. carpinifolia* var *cornubiensis* surviving. In most counties very few large elms remain, although there is considerable regeneration of young elm suckers.

In northern Britain, with an estimated population of 7 million elms, disease development has generally been much slower, although rates of spread in some cities and rural areas have been similar to those experienced in the south.

Control in southern England

In the early 1970s, a number of local authorities started control programmes, but for various reasons most were abandoned after a few years. The only long-running campaigns have been in Brighton, Hove and East Sussex. These control programmes were started in 1971, so it is now possible to review the results achieved in the past decade. Although the techniques have been refined over the years, the essentials of the programme remain the same as when control was introduced. The three main elements are: (a) sanitation felling, (b) prevention of root graft transmission and (c) restriction of movement of elm timber. Supplementary control measures include fungicide injection, and the pruning of diseased branches. The results from these control areas will be considered separately as Brighton and Hove provide examples of concentrated urban elm populations, while East Sussex is largely a rural area with a more scattered elm population.

Brighton and Hove

The total elm population for Brighton and Hove is around 30,000 and this accounts for a high proportion of the trees in the two boroughs. In Brighton there are extensive plantings of English elm *Ulmus procera* in the town centre while the Hove parks are dominated by Huntingdon elm *U.* x *hollandica*. In both towns most of the street trees are either Wheatley elm *U. carpinifolia* var *sarniensis* or Huntingdon elm. The losses through Dutch elm disease are given in Table 1, for Brighton from 1971 to 1981 and for Hove from 1974.

Table 1. Dutch elm disease losses in Brighton and Hove

Year	Brighton		Hove	
	No. of trees	Percentage of remaining population	No. of trees	Percentage of remaining population
1971	17	0.08	–	–
1972	78	0.39	–	–
1973	90	0.45	–	–
1974	28	0.14	34	0.34
1975	228	1.15	100	1.00
1976	121	0.61	100	1.01
1977	574	2.95	395	4.04
1978	266	1.41	416	4.43
1979	173	0.93	298	3.32
1980	253	1.37	310	3.58
1981	160	0.87	169	2.02

The losses prior to 1975 were low. From 1975 onwards there have been annual fluctuations, but apart from 1977 no dramatic increase has occurred. The 1977 figures were high, partly because they include some trees which were infected in 1976, but in which the symptoms were masked by the drought of that year. Since 1975 the mean annual loss for Brighton has been 1.3 per cent of the remaining population and for Hove 2.7 per cent. Despite a cumulative loss of 9.9 per cent in Brighton and 18.2 per cent in Hove, most of the important amenity plantings are still intact. In Hove, many of the losses have been in semi-woodland areas of English elm on the western boundaries of the town. The loss of these elms is only of minor consequence, for they were in difficult areas for disease control and were always a reservoir of infection threatening the elms in the town centre.

The control campaign centres on the prompt felling and destruction of diseased trees. Felling is carried out by specialist tree gangs of the Parks Departments and most diseased trees are now felled within a few days of being reported. All private trees are felled free of charge. In addition financial assistance is given to private owners in a three mile buffer zone on the western boundary of Hove. The whole felled tree is burnt; none is sold for timber or firewood. In addition, stumps are debarked down to ground level.

In recent years root graft transmission of the disease has been a major problem, accounting for a large proportion of the losses. Some attempt has been made to reduce this by trenching or injection with metham sodium.

Movement of elm timber into the area is prohibited as Brighton and Hove, together with parts of the Adur District to the west, are covered by the Dutch Elm Disease (Restriction on the Movement of Elm) Order 1978. Large warning signs have been placed at the roadside on the boundary of the control zone. Continuing problems have been created by the introduction of firewood from diseased elms felled in West Sussex. Figure 1 illustrates the spread of DED among street trees in Hove in 1979. Twenty-eight trees were infected from beetles emerging from a single stack of elm logs. This one instance accounted for 16 per cent of the trees killed by the disease in the town in that year. Publicity to this matter has been given in the local press and on radio. In 1982 there is to be a 'Log Amnesty', in which the local authorities offer to replace elm logs with suitable alternatives such as oak or beech.

The pruning out of diseased branches has often been successful, especially when restricted to trees showing only the early symptoms of the disease.

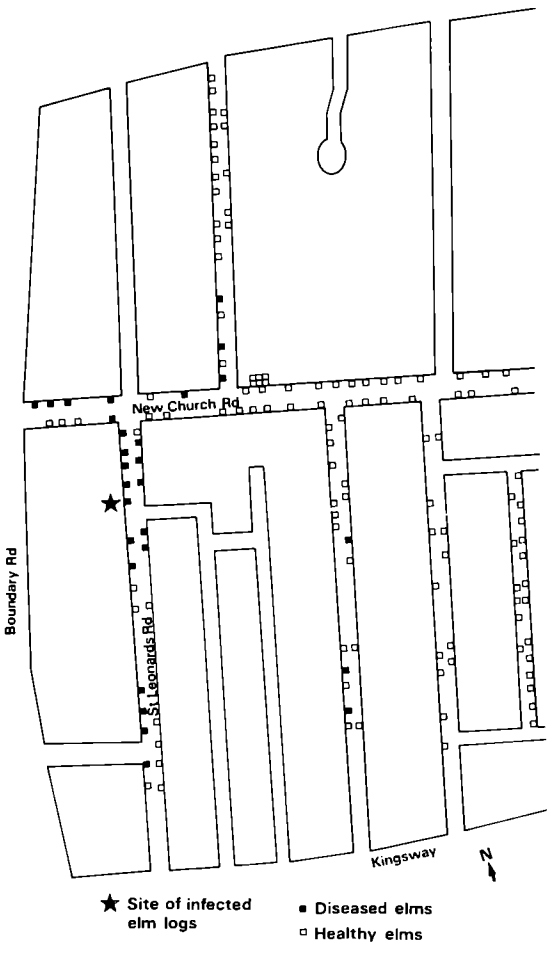

Figure 1. Spread of Dutch elm disease from an infected stack of elm logs in Hove, 1979.

In most years some 30 trees have been saved by early pruning.

Protective and curative fungicide injection with Lignasan has been carried out in Brighton for several years with variable success. Since 1977 the Forestry Commission has conducted most of its fungicide injection trials in Hove and the neighbouring town of Shoreham in the Adur District. In an experiment with Lignasan (carbenadzim hydrochloride) 100 trees were protectively injected annually from 1977 to 1981. In Shoreham, where diseased elms have not always been felled promptly, over half the injected trees died within the five-year period and 82 per cent of non-treated trees became diseased. In Hove, where prompt sanitation felling has been carried out only two control trees and one injected tree died between 1978 and 1981. These

results, illustrated in Figure 2, show that Lignasan is not effective where control programmes are lax and is unnecessary in areas which have a really effective sanitation control programme.

More encouraging results have been obtained with curative injections of Ceratotect, containing thiabendazole (TBZ) hypophosphite. Some results from experiments in Hove are shown in Table 2 (from Greig and Coxwell, 1983). Of 29 trees injected between 1978 and 1980, 24 (82.7 per cent) have recovered, four are still diseased (in 1982) and one has died, whereas 90 per cent of control trees were killed.

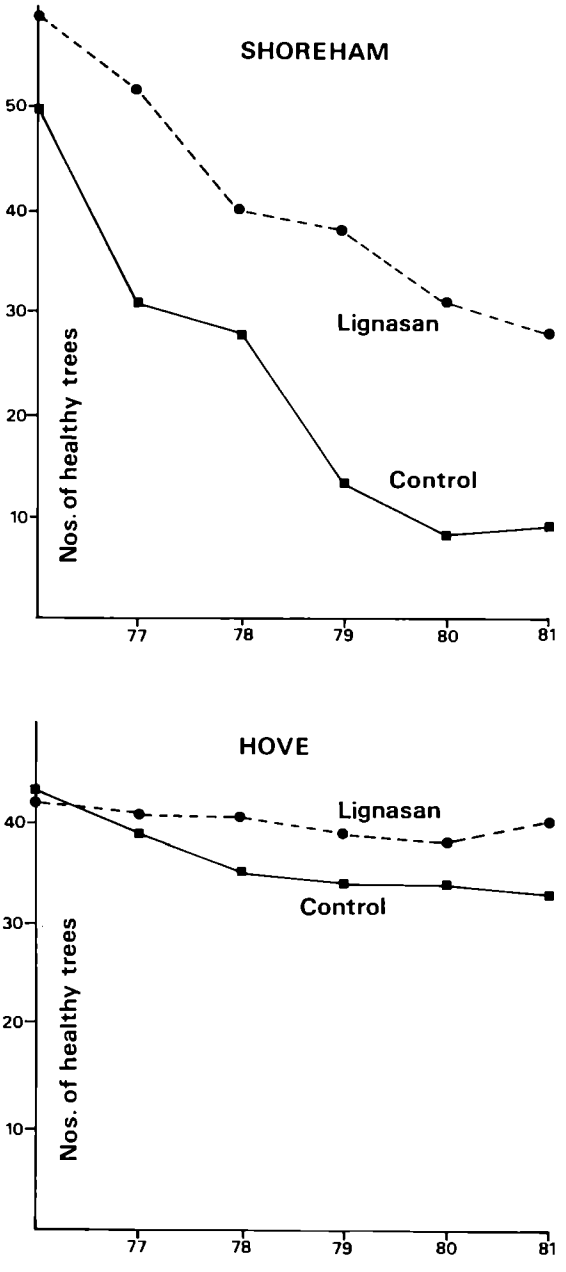

Figure 2. Results from Lignasan protective injections in Shoreham and Hove from 1977–1981.

The 'recovered' trees remained healthy in subsequent years and by 1981, vigorous new growth had virtually obscured the original diseased branches. These results show that trees can be cured of DED by Ceratotect, providing that treatment is carried out in the early stages of infection. Ceratotect will not prevent root graft transmissions or carry-over infections from the previous season.

Bio-assays from twigs collected at various intervals, showed that the fungicide was present in a high proportion of the samples during the summer and autumn following injection, and was well distributed throughout the crown. Fungicide was present in about half the samples during the following season, but little activity remained after two years. Some foliage phytotoxicity occurred in certain years, especially on pollarded Wheatley and Huntingdon elms, but generally the trees regained full foliage the next year.

A voluntary organisation, "Save-the-Elms Campaign" has played a major part in alerting the public to the importance of the local elm population, by mounting exhibitions and publicity drives. Volunteers have also been active in surveys for diseased trees and in early years carried out much of the felling of privately owned trees.

To co-ordinate policy between the local authorities involved, regular meetings are held with representatives from Brighton Borough Council, Hove Borough Council, East Sussex County Council, West Sussex County Council, Adur District Council, "Save-the-Elms Campaign" and the Forestry Commission.

The annual costs of Dutch elm disease control are currently around £10,000 for each Borough. If control was abandoned, it is estimated that the total cost of felling and removing the elms in Brighton and Hove would be in the order of £1,500,000.

East Sussex

The aggressive strain of *Ceratocystis ulmi* was first confirmed in East Sussex in 1971 and the County Council immediately began a control campaign. Approximately one third of the County was designated a DED control area. Within this area it was estimated there was a population of 80,000 predominately English elms. Most of these elms occurred in farmland and around villages, although

Table 2. Results from thiabendazole hypophosphite injection trials 1978–80

Year	Total No. of trees injected	No. of trees which recovered	No. of trees remaining diseased	No. of trees which have died
1978	4	4	0	0
1979	11	8	2	1
1980	14	12	2	0
Totals	29	24	4	1
Proportion of overall total		82.7%	13.8%	3.5%

6,000 urban elms were present in Eastbourne. Most of these elms were in private ownership and the County Countil agreed to provide sufficient funds to fell and remove all diseased elms at no cost to the owners. Revenue from the sale of timber would be offset against the County Council costs.

From 1976 to 1979, the Countryside Commission supported the control programme by giving a grant to cover half the annual costs of the campaign. The Countryside Commission grant was withdrawn in 1980 due to financial cut-backs. As the County Council was unable to provide funds for the entire programme, the control zone was reduced in area. The new inner control zone covered the main concentration of elms in the heart of the area and included the Cuckmere Valley and Eastbourne. The new control zone was slightly less than half of the old area (see Figure 3). Table 3 shows the annual losses for the new inner zone from 1971 to-date.

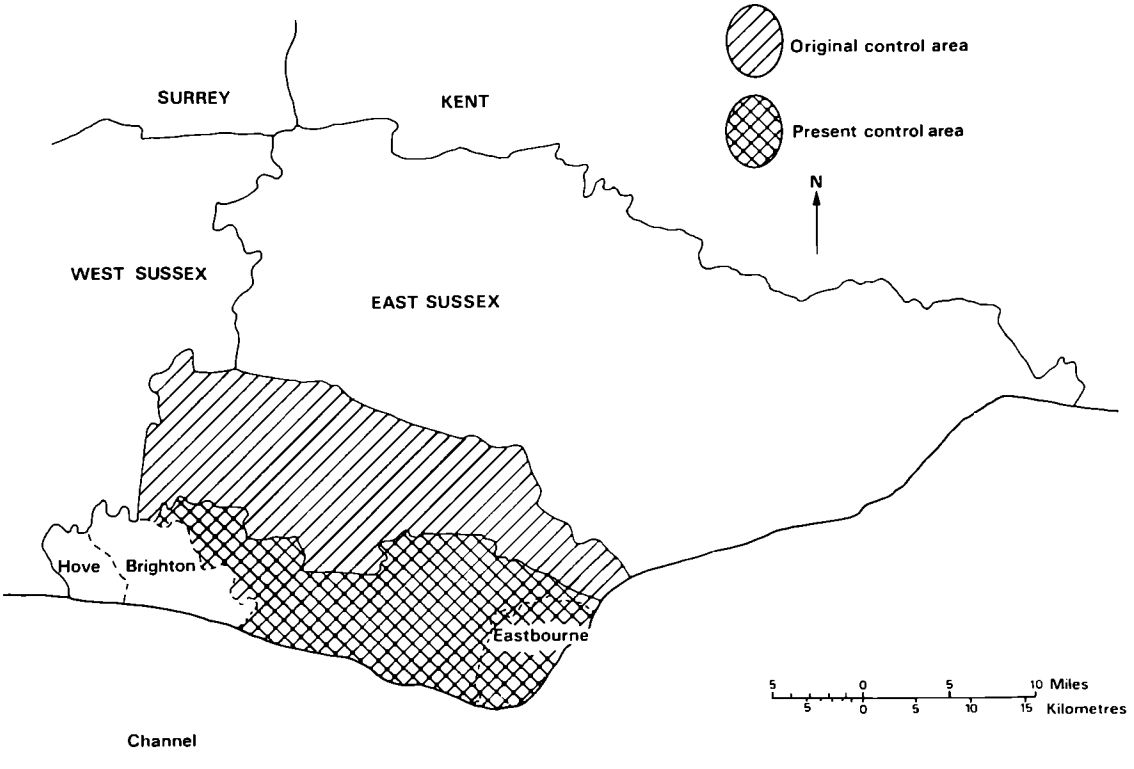

Figure 3. Map of East Sussex showing control zones.

Table 3. Number of diseased elms felled in sanitation control programmes in East Sussex inner control zone

Year	DED losses
1971	46
1972	339
1973	385
1974	680
1975	1746
1976	1009
1977	1742
1978	2507
1979	3283
1980	2721
1981	1844
	16302

There are no firm figures for the number of elms in the inner control zone. Estimates suggest that around 40 to 50 per cent of the population has been killed. Despite these high losses, the impact on the landscape has not been great and many important scenic features remain. Undoubtedly there has been considerable recruitment of young elms into the population over the past ten years, which to some extent compensates for the trees killed by DED.

The programme is controlled by a full-time DED supervisor, plus three field officers from April to November, who have area responsibilities. All felling is carried out by approved contractors and the timber is sold by tender. The cost of the programme for 1981/82 was £77,000 and the income from sale of timber £12,500.

There has been a major replanting programme complementary to the sanitation felling and up to 10,000 trees of various genera have been planted annually in the control area for the past six years.

Control in northern Britain

No comprehensive area-related survey of the elm population or of the level of disease has ever been carried out in this part of the country. Scattered surveys in parts of northern England and Scotland in which elms were particularly common, showed elm densities of around 100 trees per km^2 (Gibbs and Howell, 1974; Redfern, 1977). From these data a very tentative figure of seven million elms over 6 m in height can be suggested for northern Britain.

When the aggressive strain was first recorded in northern Britain, it was suggested that various factors might be expected to reduce the rate of disease development in this area. These included the generally smaller elm population and the preponderance of wych and Wheatley elm (Gibbs and Howell, 1974; Redfern, 1977). Attention was drawn to the possible effects of the generally cooler climate of northern Britain on the activity of the elm bark beetles. Much valuable research on this topic has now been carried out and forms the subject of a separate presentation to this meeting (Kirby, 1983).

Recently considerable interest has been aroused by the discovery that if the inner bark of a dying elm is colonised by the fungus *Phomopsis oblonga* then breeding by bark beetles is prevented (Webber, 1981). It appears that *P. oblonga* is more likely to exert an effect in northern Britain than it did in the south (Webber and Gibbs, 1983).

Data have been obtained for the years since 1977 from a number of plots containing individually marked trees, and they show that rates of disease development have generally been slower in northern Britain than further south. In 1977 six 1 km^2 plots were established in Scotland. The aggressive strain of *C. ulmi* was present in all of the plots, and there were no co-ordinated control efforts. Cumulative losses through disease increased only from 1 per cent in 1977 to 3 per cent in 1980. These losses were very small compared with the loss of trees by other causes, such as hedge clearance. Not all the plots in northern Britain tell the same story however. In one rural plot in north-west England, the cumulative loss increased from 2 per cent in 1977 to 65 per cent in 1980, and even in some urban plots where a reasonable attempt at sanitation felling has been made, losses increased during the same period from less than 1 per cent to over 20 per cent.

Northern counties of England

A number of local authorities in northern England began control programmes in the mid-1970s. In 1976 a Northern Counties Working Group on DED was established to co-ordinate the control measures in the area. The Group produced a Code of Practice for local authorities to assist the management of control programmes. Some schemes were supported by Countryside Commission grants, but this support was withdrawn in 1979. After a few years most authorities abandoned their programmes due to financial difficulties, usually when the number of diseased trees increased from a few hundred to several thousand. By 1982 only West Yorkshire, Tyne and Wear and a few towns and cities still continued with active sanitation programmes.

In cities such as Manchester and Liverpool most of the elms have now died and disease spread within these urban populations has been as rapid as that in the south. In Merseyside the aggressive strain was first recorded in 1973 and a survey in 1974

estimated that there were around 18,600 highway elms in the county (Marshall and Dawkins, 1981). Dutch elm disease became a major problem in 1974 and a programme was implemented to remove diseased elms to ensure public safety. No funds were made available to fell privately owned elms. The disease spread rapidly from 1976 onwards. It proved not to be possible to complete the annual felling programme and many diseased trees were left standing for well over a year, with disastrous consequences. Table 4 shows the numbers of trees felled annually and expenditure on DED from 1975/76.

Table 4. Elm tree losses and expenditure due to the incidence of Dutch elm disease on Merseyside (from Marshall and Dawkins, 1981)

Year	DED expenditure £	Number of trees felled
1975/76	40,000 (estimate)	600 (estimate)
1976/77	50,000 (estimate)	860 (estimate)
1977/78	150,000	2,100
1978/79	167,000	2,700
1979/80	216,000	2,400
1980/81	216,000 (estimate)	2,100 (estimate)
1981/82	250,000 (estimate)	2,000 (estimate)
Total	£1,089,000	12,760

By the end of 1982 it is estimated that less than 6,000 elms will remain and most will probably be dead by 1985. Total expenditure by 1982 will have exceeded £1 million and the final cost will be in the order of £1.75 to £2.00 million.

The majority of the Merseyside elms could have been saved by an effective sanitation felling programme, based on the Brighton and Hove model. The costs of such a programme would have probably been between £100,000 and £150,000, instead of the £1 million incurred to-date.

In contrast to Merseyside, reasonably successful campaigns have been conducted in West Yorkshire. Local authority trees are in general promptly removed and 100 per cent felling grants are now given to private owners. By following the recommended Code of Practice, annual losses in highway trees have been kept to under 3 per cent at a cost of only £23,000 for the past 6 years. A similarly successful campaign has also been carried out in Tyne and Wear. Table 5 shows the annual losses for West Yorkshire.

Scotland

The first record of the aggressive strain of *C. ulmi* in Scotland was from Glasgow in 1975 (Redfern, 1977). The hot summers of 1975 and 1976 were very favourable for bark beetle development and the disease quickly became widespread in the eastern part of the Central Lowlands (Redfern, 1977). By 1981, DED was present in most lowland rural areas, but was not significant north of Tayside. The most northerly record of the aggressive strain is from Kingussie in the Highland Region.

Table 5. Elm tree losses in West Yorkshire due to Dutch elm disease

Year	DED losses
1975	138
1976	402
1977	1200
1978	3300
1979	2000
1980	4500
1981	

Selective control mainly in urban areas is practised in most regions where the disease is present, but attempts at control have largely been abandoned in the Borders and Dumfries and Galloway Regions. Important populations of elms occur in several Scottish towns and cities. For example, there are around 26,000 elms in Edinburgh and an effective sanitation felling programme has restricted total losses to around 5 per cent since 1976. Table 6 shows the disease figures for Glasgow, Dundee and Edinburgh.

Table 6. Dutch elm disease losses in three Scottish cities

Year	Number of trees killed by DED		
	Glasgow	Dundee	Edinburgh
1975	32	–	–
1976	10	33	–
1977	18	33	123
1978	22	53	326
1979	31	31	233
1980	100	35	298
1981	80–100	36	435

The overall situation in 1982

Most of the elm populations of southern England were engulfed by the DED epidemic of the 1970s and only three areas have managed to continue with long-running control campaigns. In the north Midlands and northern England where there were better chances of control than in the south, only a few authorities still continue with control measures. In Scotland, where there are fewer defendable populations of elms, several local authorities have continued with active control campaigns.

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PAPER 4

Dutch elm disease control in the Netherlands

J. K. WATER

Dutch Plant Protection Service, Wageningen, The Netherlands

After the serious epidemic in the 1930s when huge losses of elm occurred, Dutch elm disease (DED) almost disappeared from the Netherlands in the 1950s. From that time the planting of new elm clones, of field elms and later of disease resistant clones, was undertaken. In 1972 the aggressive strain of *Ceratocystis ulmi* was found for the first time in Holland. After five years this strain was isolated from samples from all parts of the country; a new epidemic of the disease had been confirmed.

In the years before 1978 there were small sanitation campaigns in some infected areas. After 1976 it was clear that the disease had become a serious problem. Impressed by the enormous impact of the disease in southern England the government started a sanitation campaign throughout Holland. There was a fair chance of success, as that country is geographically isolated by the sea to the north and west, and by the fact that there are not so many elms in the northern part of Germany and in Belgium. The objective was to slow down the epidemic in the diseased areas, and to prevent an epidemic developing in the areas with less disease.

The campaign was based on a DED Act of the Plant Protection Law by which all wilted and dead elms and also elm logs must be destroyed or debarked. In order to obtain the help of the public and to speed up the removal of trees owned privately, 100 per cent financial support was provided by the government. The organisation was in the hands of the Plant Protection Service (PD) and the State Forestry Service (SBB). The PD marked the position of all elms outside towns on maps (1:10,000) and twice a year PD officers made a health assessment of those trees. Urban authorities were responsible for elms in their area. The SBB obtained information on wilted or dead trees and they removed them as quickly as possible.

This scheme started in 1978 on a large scale throughout the country. The goal was to remove a tree within a month of it being marked by a PD officer. But that was not easy to achieve in the beginning. There was a lot of administration, and there were not enough felling gangs in the summer period. In 1979 and 1980 it was done faster and there were more officers to inspect the felling and to ensure proper debarking. After four years there is now a good working organisation.

The results are presented in the table. The number of trees felled in towns is omitted from this table. In total, about 15–20 per cent of our elms have been lost. The average loss is highest in the size category > 40 cm diameter at breast height.

Dutch elm disease control campaign in the Netherlands

Campaign year	Number of felled trees with subsidy	Subsidy costs x F 1000	Diseased trees (% total elm population)
> 1978	?	5,997	?
1978	168,000	9,065	13.4
1979	149,000	7,850	3.4
1980	139,000	7,805	3
1981	78,400	4,715	2
	580,000 (approx)	35,432	

F = Dutch florins.

In the campaign there were many regional comparisons which showed that the basis of the campaign was effective. In regions where we started later and worked less efficiently many more trees were removed and the decrease in disease occurred later than in regions where the campaign was more efficient and started earlier. It is now possible to analyse the basic elements for a successful sanitation campaign. These include:

100 per cent subsidy on the felling costs, including trees from urban areas.

One organisation to be responsible for control (and for the law).

A DED law for the removal of all wilted and dead elms.

Strict inspection of the felling place to prevent the retention of logs with bark for use as firewood.

After four years (1982) it seems that the campaign is successful and the government has been advised

to continue in the same manner. In our opinion there are no good alternatives available. If sanitation is stopped we will lose most of our elm in a short period. The removal of dead trees for safety reasons, and also the replanting programmes, will cost large sums of money. The alternative of lowering the costs by reducing the subsidy for elms under private ownership is not likely to be so effective as individuals will be less co-operative. Another alternative is to run the campaign only in the western counties where the elm is most important. But this is also not so efficient because a lot of firewood with bark can be transported by private persons from the area without a sanitation campaign into the control zone.

Until now the campaign has been based only on sanitation and was not supported by other methods, such as trap trees or fungicide injection programmes. However the Plant Protection Service is now also involved in small trials with the trap tree technique, fungicide injections, and beetle flight observations with pheromone traps throughout the whole country. In the future there is the possibility of using a technique of biological control with bacteria and of introducing new elm clones from the resistance breeding programme.

PAPER 5

Dutch elm disease in Denmark

A. YDE-ANDERSEN
The Danish Plant Health Board

Denmark

The area of Denmark is about 43,000 km², i.e. twice the size of Wales and the same size as The Netherlands. Denmark is surrounded by the sea except to the south where the boundary line between Denmark and the German Federal Republic is about 60 km. The distance between the German north coast and the Danish south coast varies from 1 to 60 km.

The temperatures in the spring and early summer are the following in Denmark:

Month	Mean temperature °C	Average maximum temperature °C
May	11.0	*c.*14.0
June	14.5	*c.*17.5
July	16.6	*c.*19.5

The Danish elms

There are two indigenous species: *Ulmus glabra* Huds. (wych elm), by far the most common elm in Denmark, and *U. laevis* Pall. (European white elm), which is found only in a few localities. Among the introduced species, *U. carpinifolia* Gleditch (European field elm) is most common; other elm species are rare.

In the forests the elm has been considered a weed for the last two centuries and consequently has been cut down at an early stage when found. Today it usually can be found only in the fringes of the forests and in neglected forests. In the rural areas the elm is found, either alone or more commonly in mixture with other broadleaved trees, around 60 per cent of all farmsteads and houses standing in their own grounds. The elm is frequently planted in shelterbelts and often used as a roadside tree; in some countries nearly 50 per cent of the roadside trees are elms. In the towns, the elm is planted in parks and along streets; thus, in the municipality of Copenhagen about 25 per cent of the park and roadside trees are elms.

The elm bark beetles

Scolytus scolytus F. and *S. multistriatus* Marsh. have only been found in a few localities, whereas *S. laevis* Chap. has been found all over the country (see Figures 1 and 2).

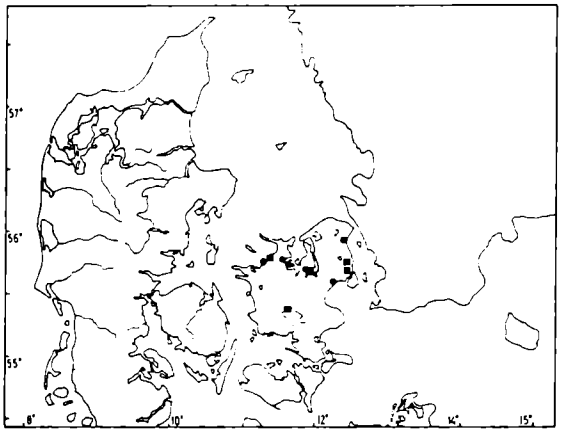

Figure 1. Occurrence of *Scolytus scolytus* F. in Denmark (After Esbjerg and Beyer, 1979)
■ Up to 1976 ● 1977 – 1978

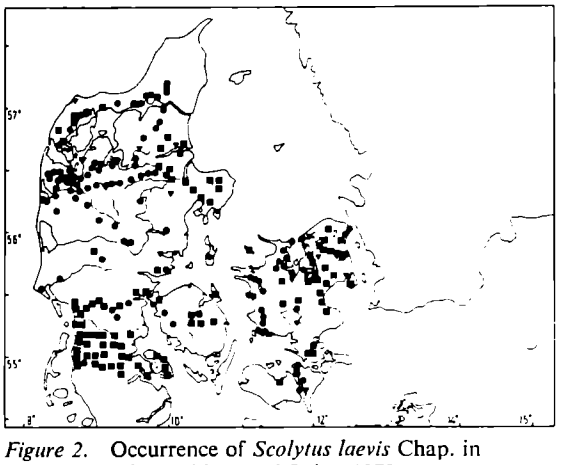

Figure 2. Occurrence of *Scolytus laevis* Chap. in Denmark (After Esbjerg and Bejer, 1979)
▼ Up to 1970 ■ 1971 – 1976 ● 1977 – 1978

Dutch elm disease

Past and present

Apart from a single outbreak near Copenhagen in 1955 Dutch elm disease had not been observed in Denmark before 1978, but discolouration of the xylem indicating attacks of *Ceratocystis ulmi* (Buism.) Moreau has been observed in annual rings formed in 1968 and afterwards. However it has not been possible to isolate the fungus from the annual rings mentioned.

From 1978 till the end of 1981 scattered attacks have been observed in almost all counties. In all the cases investigated the attack was made by an aggressive strain of the fungus, but irrespective of this the disease may show acute or chronic symptoms. When infected through root grafts, the infected elm as a rule dies in the course of one growing season; when infected by the action of elm bark beetles, the course may be the same, but sometimes elms infected in this way may, although enfeebled, survive at least 3 years.

The first severe infections appeared in about 1978, and the long-distance spread from the first infected elms has been of extremely limited extent. The local spread, mainly through root grafts between neighbouring elms has, on the other hand, in some places been extensive.

The fact that all the first infections occurred about 1978 and were caused by an aggressive strain of *C. ulmi*, combined with the geographical position of the infection centres (see Figures 3 and 4) and the prohibition against importation of unbarked elm wood which has been in force since 1956, suggests that Dutch elm disease was brought to Denmark from the south by immigration of disease-carrying elm bark beetles.

The future

There will be a permanent risk of the immigration of disease-carrying elm bark beetles from the south in years with a climate favouring the flight of the beetles. This immigration may cause scattered outbreaks of Dutch elm disease similar to those at present.

The slow long-distance spread of the disease in Denmark cannot be explained as a consequence of long distance between the elms, or of resistance in the most common elm, wych elm, but one of the following circumstances might provide some explanation:

1. The disease outbreak is only in its early stages and in a few years the situation may be similar to that presently occurring in, for example, the southern part of England.
2. The slow spread will continue in the future as a consequence of:

   either

   S. laevis being a much less efficient vector than *S. scolytus* and *S. multistriatus*. No evidence exists so far to support this assumption;

   or

   the not infrequent occurrence of *Phomopsis oblonga* (Desm.) Trav. in the bark and wood of wych elms affected by Dutch elm disease, which makes these elms less suitable as breeding places for elm bark beetles;

   or

   the frequent occurrence of *Pseudomonas fluorescens* (Trevisan) Migula in the xylem of wych elm, a bacteria which seems to inhibit or prevent the growth of *C. ulmi*.

Conclusion

Plant diseases other than Dutch elm disease and of a higher economic importance to agriculture, horticulture and forestry are found in Denmark and from an environmental point of view the elm is not an indispensable albeit valuable tree. Consequently the Ministry of Agriculture decided to abstain from the introduction of special legislation on Dutch elm disease, involving expenditure for the public as well as for the private sector. Consequently there is no compulsory control, with the exception of the prohibition against the importation of unbarked elm wood.

With the aim of delaying the spread of the disease, thus gaining time for a planned replacement of the elms with other broadleaved species, sanitary fellings are recommended.

With the aim of avoiding future disasters it is furthermore recommended to cut elms in mixed stands when necessary thinnings are undertaken and to desist from new planting of elms.

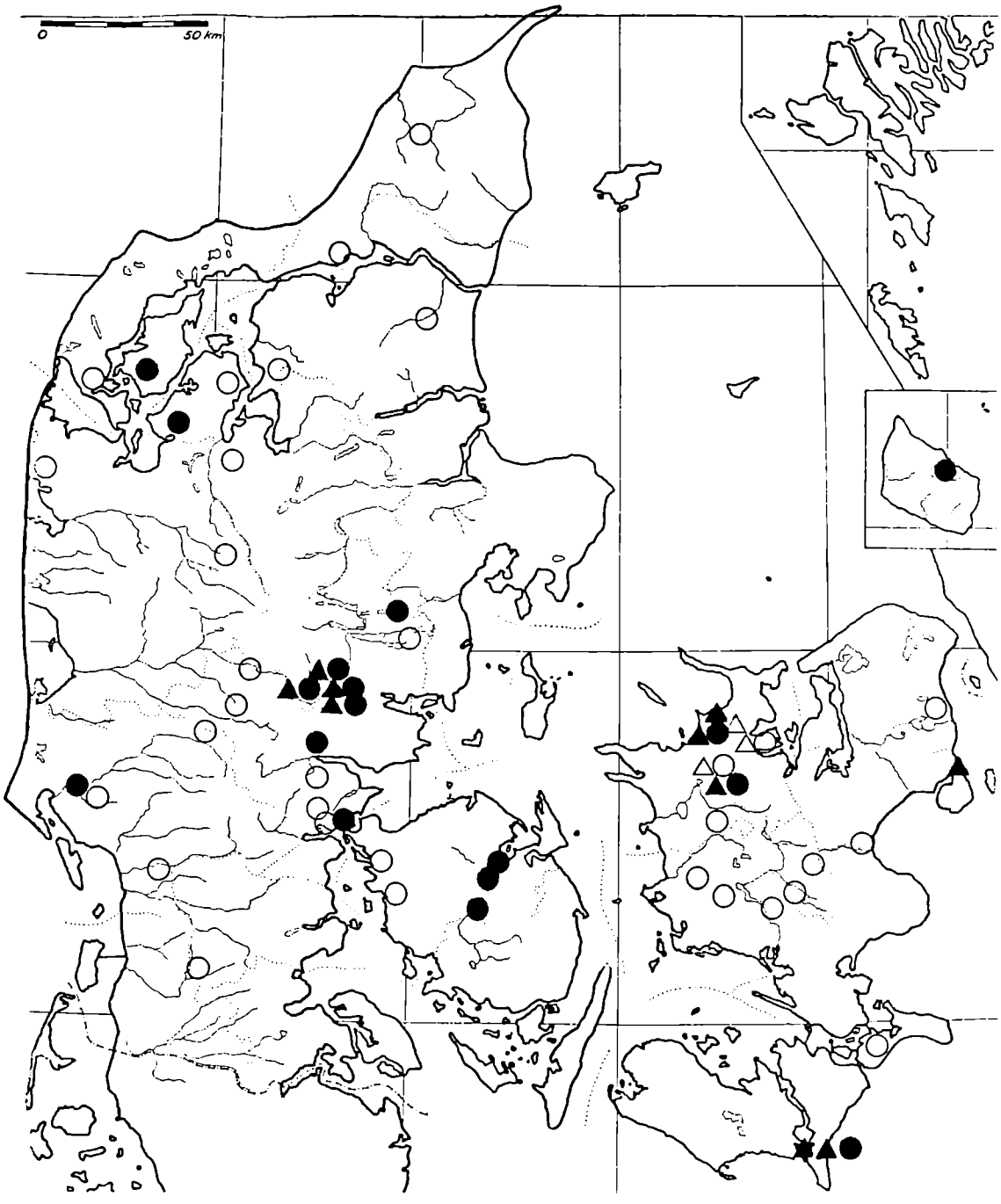

Figure 3 Occurrence of elm bark beetles in Denmark in 1981 (After Harding and Ravn, 1982)

▲ *S. scolytus* at localities with Dutch elm disease
△ *S. scolytus* at localities without Dutch elm disease
● *S. laevis* at localities with Dutch elm disease
○ *S. laevis* at localities without Dutch elm disease
★ *S. multistriatus* at localities with Dutch elm disease

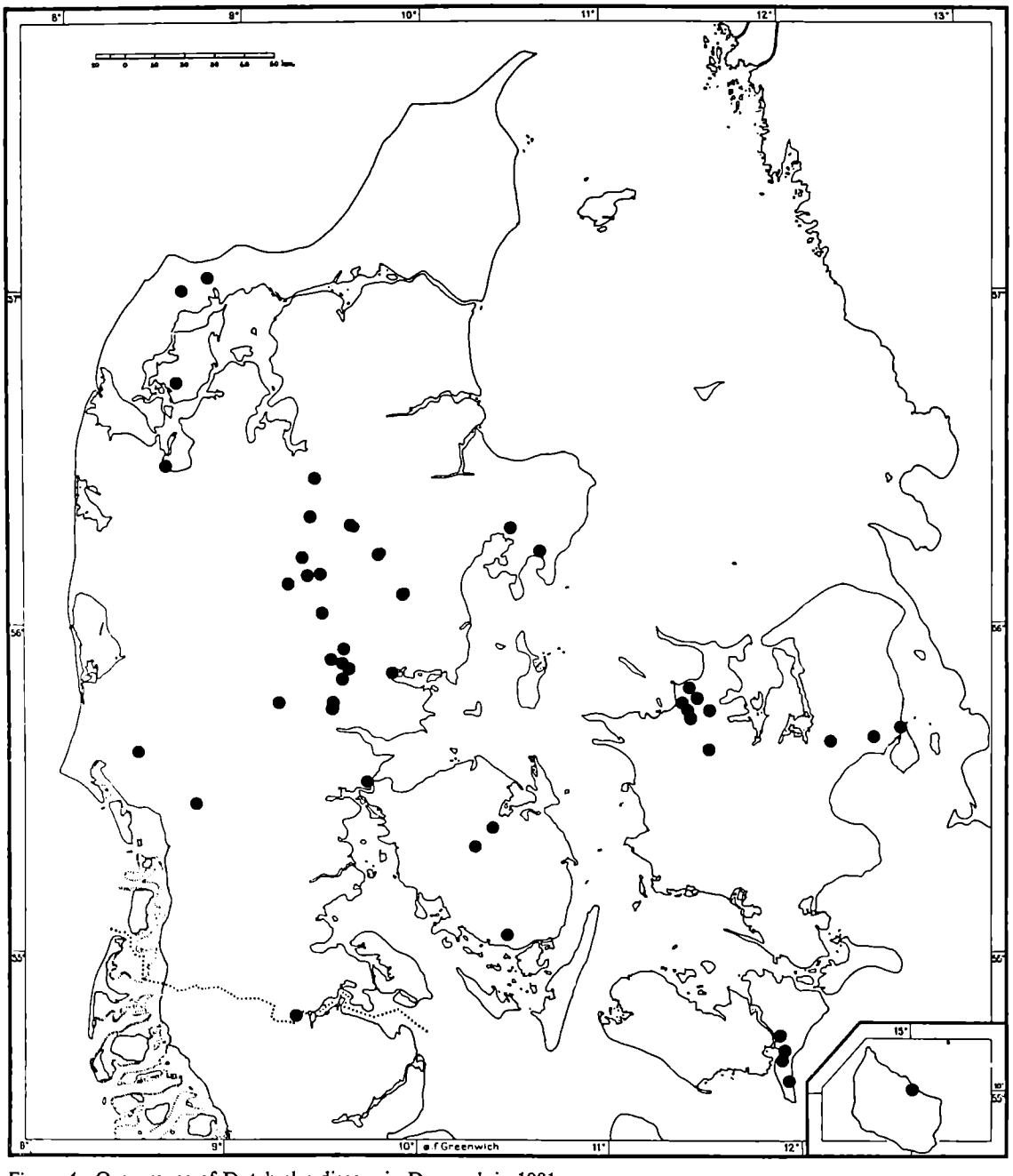

Figure 4 Occurrence of Dutch elm disease in Denmark in 1981

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PAPER 6

Evaluation of the trap tree technique for the control of Dutch elm disease in northwest England

D. P. O'CALLAGHAN and C. P. FAIRHURST
Department of Biology, University of Salford, Salford, M5 4WT, U.K.

Introduction

Dutch elm disease (DED) has decimated the elm populations of Europe and North America in recent years. The disease organism, *Ceratocystis ulmi* (Buisman) C. Moreau, is spread from tree to tree by bark beetles (Coleoptera; Scolytidae). In Britain two species have been shown to be involved, *Scolytus scolytus* (F.) and *Scolytus multistriatus* (Marsham), the large and small elm bark beetles respectively (Gibbs *et al.*, 1977). More recently another species, *Scolytus laevis* (Chapuis), has been reported from northern Britain (Atkins *et al.*, 1981). This species has been reported as a vector of DED in Scandanavia (Lekander *et al.*, 1977; Gibbs, 1978).

Because the disease is vectored by bark beetles, it can be held at tolerable levels by timely removal and destruction of diseased elms that serve as beetle breeding sites and reservoirs of disease (Gibbs, 1978; Sinclair, 1978). This process is generally known as sanitation felling and forms the backbone of any DED control programme. It has been used with success by a number of communities in the USA (Miller *et al.*, 1969) and in Britain (Greig and Gibbs, 1983 this volume and Riley, 1983 this volume).

Recent work at the State University of New York has led to the development of the **trap-tree technique** to augment and enhance sanitation procedures (O'Callaghan *et al.*, 1978). Briefly, this technique involves the killing of elms with the arboricide cacodylic acid and baiting them with pheromone to induce mass attack by *S. multistriatus* whose brood fails to develop within the treated trees (O'Callaghan *et al.*, 1980). Thus diseased elms, usually liabilities to DED control programmes, can be turned into assets capable of absorbing large numbers of beetles and preventing their reproduction. This paper describes the background to the technique and experiments to test the applications of this technique to the situation in northwest Britain.

Materials and methods

Study areas

Two sites in Merseyside County were selected for the trials; (A) the southern part of the City of Liverpool and (B) the town of Southport in the Metropolitan Borough of Sefton, 26 km north of Liverpool (Figure 1). Each site encompassed about 6.5 km^2 and was divided into approximately equal treatment and check areas separated by a buffer zone about 0.8 km wide. The two areas jointly contained about 3,000 elms of which 1,200 were diseased or dead at the beginning of 1980.

Tree treatment

In 1980 diseased elms, (more than 30 per cent of the crowns wilting), were treated with cacodylic acid (RAD-E-CATE 35(R))*, and in 1981 with either cacodylic acid or monosodium methanarsonate (SILVISAR 550(R)† or VICHEM 120 SILVICIDE (R))*. The chemicals, diluted or undiluted, were applied by pressure injection into the bole, or topically to axe-frills at waist level (1 m). No frills were used during 1981. No attempt was made to quantify the amount of chemical applied, but the frills were filled twice to the point of run-off. Between 1,000 and 2,500 ml of chemical were applied to each tree by pressure injection.

One hundred and thirty seven elms were treated in 1980 and 87 in 1981. In 1980 some of the trees were baited with components of the aggregation pheromone of *S. scolytus*, 4-methyl-3-heptanol and (-)-limonene (Blight *et al.*, 1980) dispensed separately by diffusion from 2.5 ml polyethylene snap top vials. In 1981 all trees were baited with 4-methyl-3-heptanol only, released from CONREL(R)‡ hollow fibre dispensers as used in the United States (Cuthbert and Peacock, 1978; Lanier, 1981). Individuals of all common elm types were treated: *Ulmus procera* (Salisb.) (English elm); *U. glabra* (Huds.) (wych elm) and *U. carpinifolia* var *sarniensis* (Loud.) Rehder (Wheatley elm).

* Vineland Chemical Company Inc., Vineland, New Jersey, U.S.A.
† TSI Company, Flanders, New Jersey, U.S.A.
‡ Albany International, Needham Heights, Massachusetts, U.S.A.

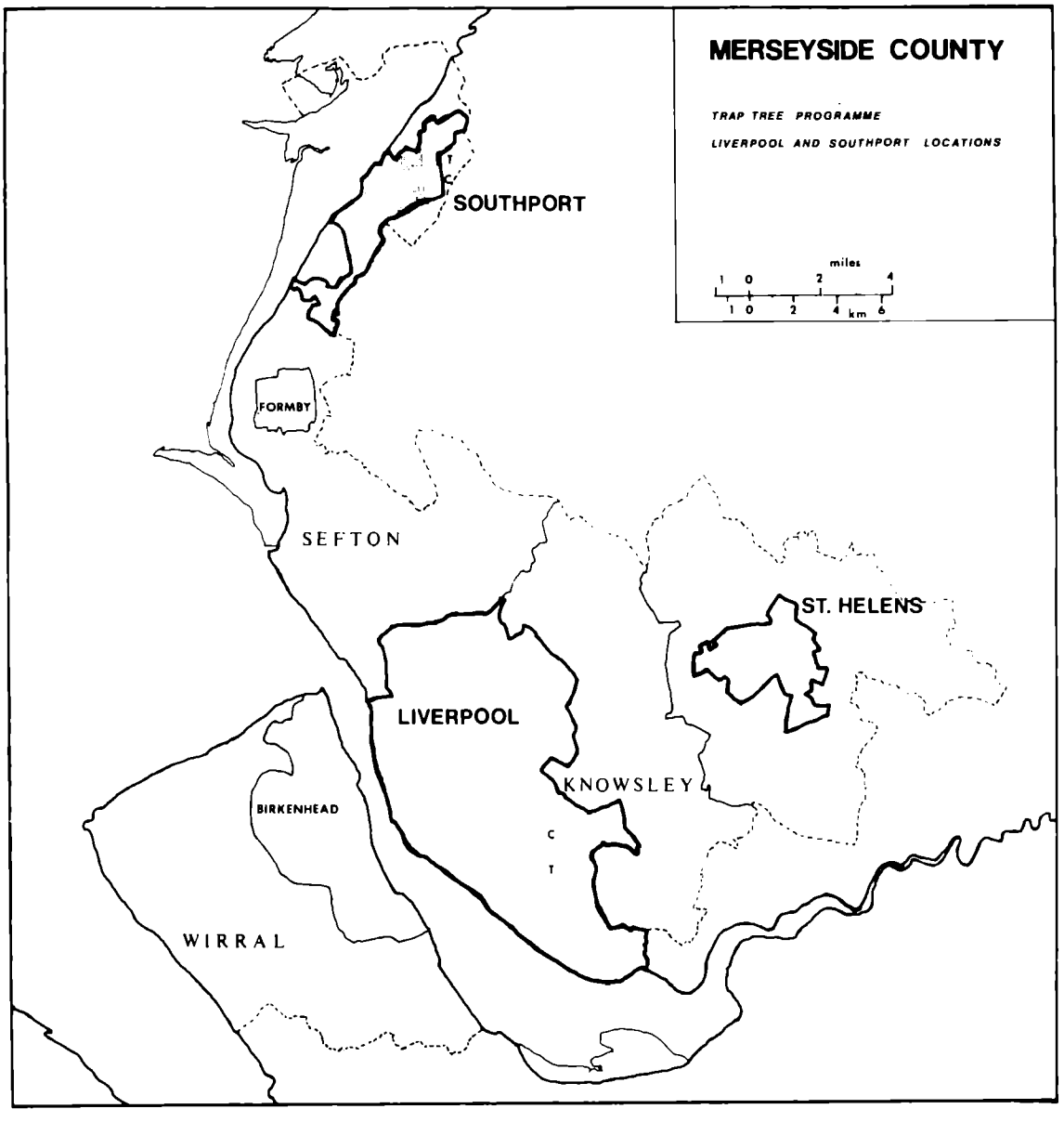

Figure 1. Map of Merseyside County showing, shaded, the treatment (T) and check (C) areas within the Liverpool and Southport sites of the trap tree trials in 1980 and 1981.

Sampling procedures

Sampling schemes were devised to evaluate the success of the treatments regarding the attraction of beetles to the treated trees, and regarding the inhibition of beetle development within the treated trees. Trees were felled and a proportion of the bark surface area (BSA) examined (about 10 per cent during 1980 and between 5 and 10 per cent during 1981). Discs 10–20 cm long were cut at one or two metre intervals from the bole and branches to the point where they were less than 5 cm in diameter. The numbers of attacks (penetrations to sapwood) and brood/maternal galleries were assessed and the number of live larvae determined by bark dissections. These data were converted to numbers per square metre (m^2) of BSA.

A number of untreated control trees close to both treatment areas were felled and sampled in a similar manner. In 1980 only Wheatley elms well-infested with beetles were studied in Liverpool, but in Southport a more random selection of trees was available for analysis. In 1981 control trees were selected during June and July (at the time of treatment) in an effort to equate the exposure to beetles of both treated and untreated elms. Trees in which the disease caused rapid and almost total (90 per cent) wilt over a short (2 week) period were selected as controls, as their decline was as rapid as that caused by cacodylic acid and monosodium methanarsonate treatments.

Most of the trap-trees were attacked by both *Scolytus scolytus* and *S. multistriatus* and in 1981 it was decided that the attacks made by both species should be recorded separately.

Dutch elm disease rates

The DED rates were assessed by a survey listing all elms in each location, first in May and again in August. Symptoms that appeared before July 1 and which were extensive were considered to be previous year infections. Symptoms that appeared after July 1 and which were restricted to single limb systems were generally considered to be current year infections.

Results

Within 48 hours of injection with cacodylic acid the trees began to wilt showing 100 per cent crown kill within one week. Tree death followed rapidly, usually within three weeks. The sub-cortical tissues became dry and mottled in appearance soon after injection, and became completely discoloured and very dry any time from three weeks post injection onwards. This brown discolouration is almost invariably due to the action of the saprophytic fungus *Phomopsis oblonga* (Desm.) Trav. (Gibbs, personal communication) that colonizes the inner bark of dying elm trees (Webber, 1981). Sometimes, if the chemical does not distribute evenly within a tree only parts of the tree die and strips of living tissue may be found between the dead portions, but this eventually (over the summer) dies also. The effects of monosodium methanarsonate seem to be identical to those of cacodylic acid. The chemicals do not move into any parts of a tree that were dead or colonized by beetles prior to injections.

In general there were more beetle attacks on all trees in 1980 than in 1981. There seems to be a clear indication that control trees were attacked more readily in both years despite the difference in beetle abundance (Tables 1, 2 and 3). The most marked effects were in wych elm in 1980 and on English elms in Liverpool in 1981. Here the ratios of attack on control versus treated trees were 6:1 and 9:1 respectively.

Table 1. The number of attacks and the number of larvae per maternal gallery on cacodylic acid treated and untreated trees in Liverpool and Southport during 1980. The figures represent attacks by both the large and small elm bark beetles.

Elm species/ type of treatment	Number of trees sampled	Average number of attacks/m²	Number of larvae/ gallery
Wheatley			
Treated	17	45.6[a]	2.17[a]
Control	11	123.5[b]	14.18[b]
English			
Treated	10	40.8[c]	4.71[c]
Control	7	34.5[c]	8.65[c]
Wych			
Treated	17	12.9[d]	2.23[d]
Control	5	78.6[e]	15.24[e]

Within each species (i.e. for each pair of figures, read vertically) totals followed by different letters are significantly different; $P < 0.05$. Totals followed by the same letter are not significantly different.

Table 2. The number of attacks and the number of larvae per maternal gallery of *Scolytus scolytus* (*Ss*) and *S. multistriatus* (*Sm*) on cacodylic acid treated and untreated trees in Liverpool during 1981.

Elm species/ type of treatment	Number of trees sampled	Average number of attacks/m²		Number of larvae/ gallery	
		Ss	*Sm*	*Ss*	*Sm*
Wheatley					
Treated	8	2.7[a]	1.4[a]	1.69[a]	2.05[a]
Control	4	14.1[b]	0.9[a]	15.34[b]	15.14[b]
English					
Treated	6	1.6[c]	1.4[b]	0.64[c]	0.71[c]
Control	4	15.9[d]	10.8[c]	8.10[d]	11.33[d]
Wych					
Treated	4	0.7[e]	1.4[d]	0.23[e]	0.08[e]
Control	3	10.4[f]	0.0[e]	13.63[f]	0.00[f]

Within each species (i.e. for each pair of figures, read vertically) totals followed by different letters are significantly different; $P < 0.05$. Totals followed by the same letter are not significantly different.

Table 3. The number of attacks and the number of larvae per maternal gallery of *Scolytus scolytus* (*Ss*) and *S. multistriatus* (*Sm*) on cacodylic acid treated and untreated trees in Southport during 1981.

Elm species/ type of treatment	Number of trees sampled	Average number of attacks/m²		Number of larvae/ gallery	
		Ss	*Sm*	*Ss*	*Sm*
Wheatley					
Treated	3	3.9[a]	0.0[a]	3.63[a]	0.00[a]
Control	2	3.5[a]	0.1[b]	10.08[a]	22.09[b]
English					
Treated	6	0.8[b]	0.1[c]	2.91[b]	1.10[c]
Control	3	0.8[b]	0.01[c]	9.53[b]	11.67[d]
Wych					
Treated	7	1.4[c]	0.1[d]	0.84[c]	1.01[e]
Control	2	0.5[c]	0.0[e]	11.95[d]	0.00[f]

Within each species (i.e. for each pair of figures, read vertically) totals followed by different letters are significantly different; $P < 0.05$. Totals followed by the same letter are not significantly different.

Table 4. The Dutch elm disease rates* in the Merseyside test areas.

Year	Liverpool areas		Southport areas	
	Check	Treatment	Check	Treatment
1979	17	19	39	23
1980	77	32	33	19
1981†	44	16	28	12

*Rate = Percentage of trees healthy at the beginning of the year that became diseased during the year.
†1981 = Subject to confirmation in spring of 1982.

Analysis of the two beetle species separately indicates that this effect is due predominantly to the activity of *S. scolytus*. From the data available for *S. multistriatus* it seems possible that it has a rather different pattern of behaviour as its density of attack on control trees was higher than that on treated trees in only two of six comparisons (Tables 2 and 3).

Brood galleries were formed equally well in both treated and control trees, but those in control trees were more successful as seen by the numbers of progeny produced per gallery (Tables 1, 2 and 3). In all treated trees in each location throughout both years fewer progeny were recorded per gallery than in the control trees. These differences in the numbers of larvae per gallery between treated and control trees were statistically significant (χ^2; $P < 0.05$) in most instances. The brood productivity of *S. multistriatus* was significantly lower in all treated trees compared with control trees (Tables 2 and 3).

In Southport the Dutch elm disease rates showed a consistent decline throughout the trial period. This trend was not evident in Liverpool, where an increase was recorded in 1980, due probably to a backlog of sanitation felling (Table 4).

Discussion

In agreement with the results of the American trials of the trap-tree technique (O'Callaghan *et al.*, 1980), the number of larvae per maternal gallery is substantially reduced in cacodylic acid treated trees in Merseyside (Tables 1, 2 and 3). In many respects however, there are major differences between the British and American trial results. First, O'Callaghan (1982) has shown that of 302 dead elms examined in the Merseyside area, only 20 per cent were fully utilized by beetles for breeding and over 50 per cent had no beetle galleries at all. This large between-tree variation poses difficulties for experimental design and interpretation of results, and a third year of trials is needed for thorough replication. Secondly, differences in climate mean that the timing of cacodylic acid is significantly more difficult in Britain. In the U.S.A., elm bark beetle flight periods are easier to predict and diseased elms can be treated immediately before or during beetle flights, which is ideal for dense attack (Lanier, personal communication). In northern Britain however, there were relatively few flight days (days when the maximum temperature equals or exceeds 22°C). These are difficult to predict and average only 20 between May and September with considerable year to year variation (Fairhurst and King, 1983 this volume). It happens therefore, that some weeks may elapse betwen the treatment with cacodylic acid and the onset of climatic conditions suitable for beetle flights.

An additional factor in the Merseyside trials is the presence of the saprophytic fungus *Phomopsis oblonga*. This fungus can make the inner bark unsuitable for beetle breeding (Webber, 1981). There is also evidence to suggest that *P. oblonga* can limit beetle attacks to a particular tree or parts of trees (O'Callaghan, 1982). The results of the present study suggest that trees treated with cacodylic acid are colonized rapidly by *P. oblonga* and the characteristic zone lines (Webber, 1981) were recorded on more than 90 per cent of samples taken in 1981. In addition it has been shown that cacodylic acid promotes the growth of *P. oblonga in vitro* (Edwards, 1981). It seems reasonable to suggest therefore, that a delay in the conditions favouring beetle flight following treatment, results in treated trees being

rendered unattractive to flying beetles by the action of *P. oblonga*, despite the addition of pheromone baits.

The relative lack of attractiveness of treated trees to flying *S. scolytus* is seen in the experimental results, particularly those of the Liverpool site (Table 2). The effect on *S. multistriatus* is less obvious apart from that on the English elms in the Liverpool site (Table 2). *Scolytus multistriatus* normally colonizes the thinner branches (Kirby and Fairhurst, 1981) and seems to respond quicker to diseased elms in earlier stages of decline than does *S. scolytus* (O'Callaghan and Kirby, personal observations).

The prognosis for use of the pheromone-baited trap-tree technique in DED control programmes in northern Britain is uncertain. Treated trees clearly do not absorb large numbers of beetles but they do prevent beetle reproduction. Treatment with cacodylic acid can neutralize a tree from a beetle stand point, as the colonization of inner bark by *P. oblonga* following treatment effectively prevents beetles breeding in these trees, and this should help to reduce the numbers in the next generation. Such neutralization of trees could therefore help to reduce disease spread, particularly in areas in which elms are not readily accessible for felling or where available staff must concentrate on curative injections, pruning of specimen elms and general tree work.

Acknowledgements

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PAPER 7

The ecology of elm bark beetles in northern Britain

S. G. KIRBY, and C. P. FAIRHURST
Department of Biology, University of Salford, Salford, M5 4WT, U.K.

Introduction

This paper is a synopsis of research carried out over the last three years into the biology of elm Scolytidae with particular regard to their development in northern Britain and in relation to Dutch elm disease (DED) transmission. British research during the early 1930s (Fisher, 1931, 1937) and throughout the 1960s (Beaver, 1966, 1967) seems to have concentrated mainly on the general biology of the large elm bark beetle *Scolytus scolytus* (F.) breeding in English elm *Ulmus procera* Salisb. in southern England.

By early 1979, when the University of Salford's current work programme started, the elm population of the south of England had already suffered major losses due to DED. Since the arrival of the aggressive form of the pathogen *Ceratocystis ulmi* (Buism.) C. Moreau, in the late 1960s (Gibbs *et al.*, 1972; Gibbs and Brasier, 1973; Brasier and Gibbs, 1973) these losses had amounted to between 80 and 90 per cent of an elm population estimated at approximately 23 million trees (Gibbs and Howell, 1972). The situation in northern Britain was, however, markedly different with most of the 5 to 7 million elms still remaining. Since the arrival of the aggressive strain of *C. ulmi* in this region in the early 1970s losses had not mirrored the southern experience. Major losses (>10 per cent) were restricted to that area south of an imaginary line from the River Mersey on the west coast to the River Humber on the east (Kirby *et al.*, 1981). In 1979 there was little reason to suppose that factors controlling disease incidence were the same north and south. The research programme of the subsequent three years has been primarily concerned with studying the effect of latitude on bark beetle biology and ecology.

Background and objectives

The tendency for lower ambient temperatures to occur in northern Britain means that vector performance could be adversely affected in many ways. English elm had been planted widely in the south as a hedge and shelter-belt tree. This together with the formation of a common root system between trees allowed beetle feeding to initiate a chain of infections (Brasier and Gibbs, 1978). Disease rates could, therefore, increase rapidly in areas harbouring low and/or sparse beetle populations during the early phases of the epidemic. This is entirely different to the northern situation where wych elm *U. glabra* Huds. although the dominant elm, differs in its distribution.

Wych elm is a tree native to Britain and is planted as a specimen tree in parks, gardens and open spaces, particularly in the north. Wych elm is limited in its distribution to land below approximately 180 metres. In northern urban locations Wheatley (Jersey) elm *U. carpinifolia* var *sarniensis* (Loud.) (Rehder) has been planted widely. In certain instances (parts of Merseyside and Greater Manchester) Wheatley elms have been planted to the exclusion of other tree species. The response of elm bark beetles to different hosts, therefore, represented an important area for investigation.

Vector distribution figured high on the list of priorities for study. It was known that both *S. scolytus* and the small elm bark beetle *S. multistriatus* (Marsh) were common in the south during the main phase of the epidemic there (Gibbs, 1971). Preliminary postgraduate studies at the University of Salford prior to 1979 had indicated that *S. multistriatus* was comparatively scarce in many northern counties of England, and Redfern (1977) could find no record of it in Scotland. *Scolytus scolytus* was, however, common in the Borders region of Scotland during the late 1970s (Crowson, 1976; Redfern, 1977). At the start of this work programme these two species were the only Scolytidae thought to be involved in disease transmission.

Methods

In order to allow direct comparison with previous beetle population studies the methods of Beaver (1967) were used as the basis for experimental work described here. This involved monitoring infested elm billets (120 cm in length, 5 to 20 cm in diameter) and cutting samples at regular intervals of time in

order to reveal the stages present throughout the season. The major differences between this study and Beaver's pre-epidemic work centre on the use of three host types deployed at experimental sites throughout Britain.

Disc samples were cut from infected billets of either English, Wheatley or wych elm. During the first season (1979/80) these were taken at monthly intervals. In the second season (1980/81) sample frequency was increased to give cuttings every two weeks throughout the spring, summer and autumn months. Each sample disc measured 10 cm in length and therefore the total area sampled on each occasion and finally, for the season as a whole, can be calculated. A total of 18 logs of each elm type was used at each site, samples being cut from 12 of these on each visit.

Samples were returned to the laboratory and the bark peeled away to reveal the galleries and immature stages present. The results presented and discussed represent accumulated data for three seasons work at seven sites: two in Greater Manchester, two on Merseyside, one in Cheshire, one in Devon and one in Lothian, Scotland.

Results and discussion

The results obtained will be presented in a format dictated by the biology of elm scolytids in relation to disease transmission. For this reason Figure 1 is provided as a diagramatic representation showing some of the features now realised as playing an important role in beetle ecology as it affects the relationship between vector, host and pathogen.

1. *Species of elm bark beetles*

Until very recently it was assumed that only two scolytid species were involved in disease transmission within the British Isles (excluding the Channel Islands). These are *S. scolytus* and *S. multistriatus*. Another species *Pteleobius vittatus* (F.) known to exist in moribund elms in southern England has not been considered as a vector, even though it infests narrow diameter branches (2.5 to 6.0 cm), for adults are not known to feed on twig crotches (Fransen, 1939; Beaver, 1967). In addition the adults of *P. vittatus* emerge with mature sexual organs (Beaver, 1967) and therefore maturation feeding is not necessary. Perhaps the most striking difference between *P. vittatus* and other British elm bark beetles is that adults are able to overwinter along with larvae.

More recently a third species has been added to the vector list, at least in northern Britain. This is *S. laevis* (Chap.) recorded officially for the first time in Britain infesting wych elm in the Liverpool region (Atkins *et al.*, 1981). Adult specimens have since been collected from several localities on Merseyside, from Halton (Cheshire), Peterlee, Co. Durham and from Musselburgh, near Edinburgh, Scotland. Crowson (1982, Glasgow Naturalist, in preparation) has also confirmed *S. laevis* amongst specimens collected from the Glasgow area. Adults of *S. laevis* are known to feed on healthy elm twigs. Spessivtseff (1921) believed adult feeding to be essential for newly emerged adults and for the present time it must be assumed to be a potential vector. Practically nothing is known of the biology of *S. laevis* in Britain although Bejer-Petersen (1976) has studied it in detail in Denmark along with *S. scolytus*, *S. multistriatus* and *S. pygmaeus* (F.).

The most northerly records so far obtained for the British distribution of *S. multistriatus* are Southport, Lancashire on the west coast and Driffield, Humberside on the east. Although it is highly unlikely that these are the exact northern limits it may be accepted that *S. multistriatus* is not as common or widely distributed in northern Britain as *S. scolytus*. However, *S. multistriatus* may be extending its range northwards with the increased availability of suitable host material. It would not be unusual to find that *S. multistriatus* is limited to more southerly latitudes; the distribution of many bark beetle species is limited in this respect (Swaine, 1925).

If we consider those elm areas known to have suffered or to be currently experiencing high DED rates in Britain we find established populations of *S. multistriatus*. This species is an aggressive feeder and one known to possess a powerful chemical attractant (pheromone) capable of inducing high density attack during breeding colonisation (Peacock *et al.*, 1971). It is also known that twig-crotch feeding and therefore disease transmission is linked to courtship and mating (Svihra and Clark, 1980). In northern areas where weather conditions do not favour the establishment of *S. multistriatus* populations a slower rate of disease advancement could be experienced. If, however, *S. laevis* is present, the disease rate may not decline as this species is known to vector the disease successfully throughout much of northern Europe (Lekander *et al.*, 1977) and is presumably better acclimatised for survival under northern conditions.

2. *Beetle emergence and dispersal*

It has previously been reported that beetle flights may be severely restricted by ambient temperatures (Fransen, 1939; Von Keyserlingk 1980, 1981). In view of the more adverse climate of northern Britain it seems that suitable days for flight are few in number. Early work by Fransen (1939) suggested

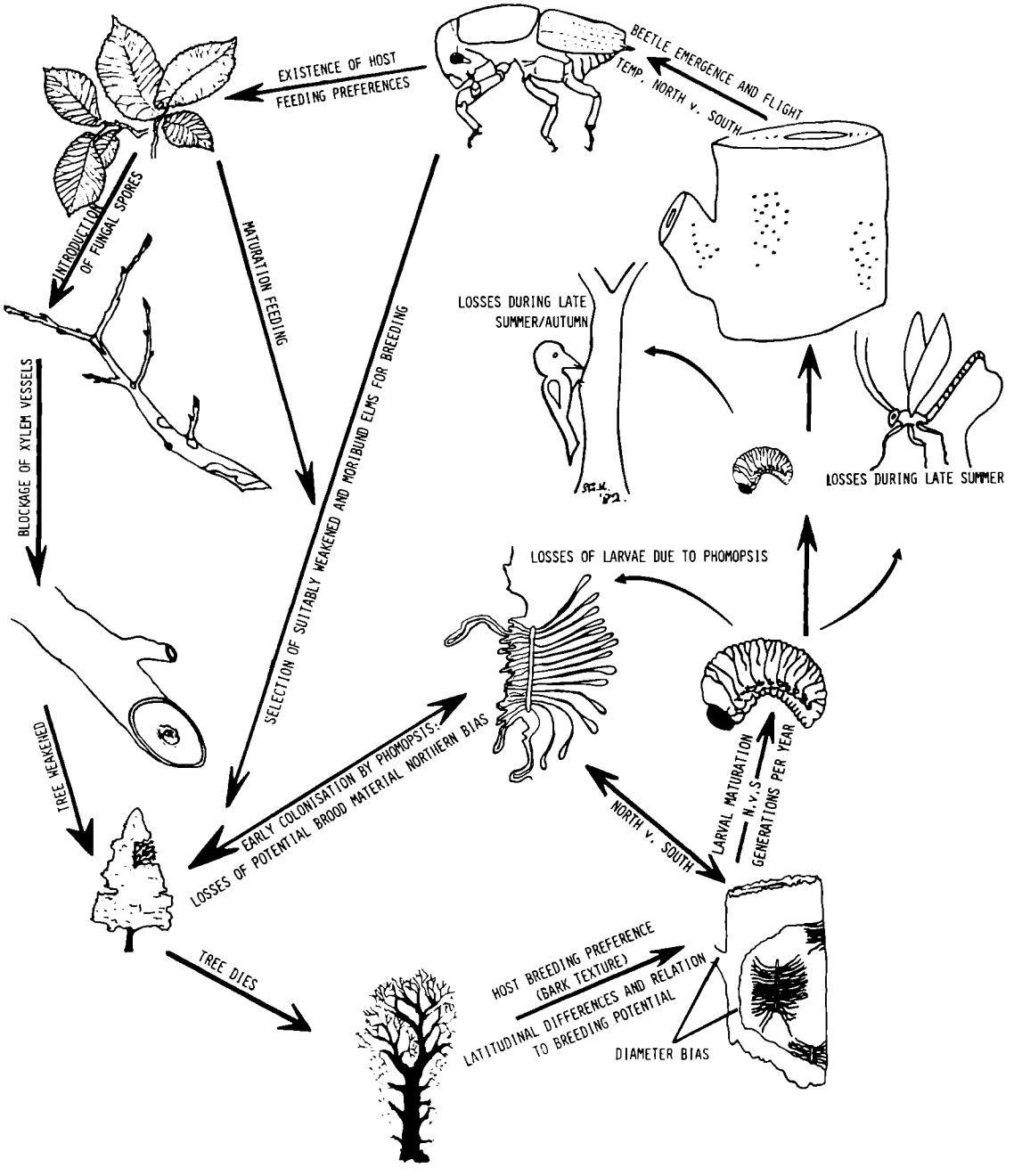

Figure 1. Diagrammatic representation of the seasonal history of *Scolytus scolytus* (F.)

an emergence threshold of 17.5°C for *S. scolytus* and a flight threshold of 20°C. More recent studies (Water, 1981 unpublished) using the same species show flight to be restricted to temperatures between 15 and 31°C with 87.5 per cent of beetles flying at temperatures above 21°C.

The tendency for warmer summers and milder winters in southern England allows for an emergence of *S. scolytus* from late May onwards through June. A second emergence, the progeny of the first, generally occurs during August and September. First flights of *S. multistriatus* follow some 2 to 3 weeks after *S. scolytus* and a second generation has been observed in English elm logs located in Devon during 1979. Because of the considerable variation in temperatures from one year to the next there is little to be achieved by presenting specific dates. It is sufficient to summarise vector emergence patterns as being governed by many factors the most influential of which are the onset and maintenance of favourable spring temperatures. More will be said of this in relation to adult feeding.

3. *Adult feeding*

Fisher (1937) concluded that although the sexual organs of newly emerged *S. scolytus* were not mature, feeding on healthy elm twigs was not an obligatory behavioural component. Adults would often complete sexual maturation whilst constructing galleries. This view is endorsed and indeed certain laboratory investigations have been hampered by the immediate re-entry of newly emerged adults of both *S. scolytus* and *S. multistriatus* into recently vacated logs. Lanier *et al.*, (1976) found that *S. multistriatus* adults in contact with pheromone odours will enter moribund elm wood directly without twig-crotch feeding. It is most likely, therefore, that twig-crotch feeding on healthy elms is environmentally induced.

This view is based on the known importance of moisture to elm bark beetle survival (Von Keyserlingk, 1980). It is suggested that the overriding need to obtain both water and nutrients is the driving force behind twig-crotch feeding. Von Keyserlingk (1980) found that *S. scolytus* adults lose water rapidly with death occurring after a loss of 20 per cent in body weight. However, major losses of water occur *after* death therefore Von Keyserlingk suggested nutrient rather than water deficiency as the key factor.

Adverse weather providing sub-threshold flight temperatures prevents beetles from locating and colonising moribund elms once emergence has taken place. Feeding must be initiated if the individual is to survive. This theory also hinges on the marked difference between emergence and flight thresholds set at 16° and 20°C respectively for *S. scolytus* (Von Keyserlingk, 1980) and supported by recent field trapping data in Holland (Water, 1981, unpublished) where the majority of beetles were caught at temperatures above 21°C. Due to the nature of the medium in which development occurs it is not sufficient simply to consider ambient air temperatures.

Wallace and Beard (1940) carried out a series of thermocouple experiments using living elm trees approximately 15 cm in diameter. They found that under bark temperature continued to rise, in spite of comparatively uniform air temperatures, because of heat absorption. This temperature increase could be responsible for sub-cortical temperatures within the log 15°C higher than atmospheric ambient. Under these conditions large numbers of beetles could emerge in a very active state only to find the surrounding air temperatures too low for sustained flight.

4. *Host feeding preferences*

One of the most interesting of behavioural responses related to feeding is the bias shown by adults to a particular host species. Since this work was carried out on a joint basis with the Forestry Commission it will be dealt with in a separate paper (Webber and Kirby, 1983, this volume).

5. *Colonisation and host selection*

(*Unless specified otherwise, results presented in this section refer to S. scolytus.*) Whether following directly after emergence from dead elms or after twig-crotch feeding on healthy trees beetles must locate and infest elms suitable for breeding. Results have consistently shown that colonising beetles exhibit a bias towards selection of English and Wheatley elm in preference to wych elm. During the 1979 and 1980 seasons' experiments beetles attacked logs cut from English elm (situated at four separate sites) between one and three weeks prior to those cut from either Wheatley or wych elm at the same location. Final colonisation densities, however, do not show this bias continuing through the season. Table 1 shows the number of maternal galleries recorded in samples of each elm type for logs situated at the Cheshire site during 1980. Although English elm logs were attacked first during the third week of May the number of galleries present in Wheatley elm by early June exceeded those in English elm. The number of galleries recorded in wych elm log samples remained low throughout the season.

During the first season's experimental programme logs had been arranged in three adjacent species rows. Using this experimental design the role of

Table 1. Maternal gallery totals for all sample discs cut between June and October 1980 from English, Wheatley and wych elm in Cheshire.

Month \ Host	English elm		Wheatley elm		Wych elm	
	S.s.	S.m.	S.s.	S.m.	S.s.	S.m.
June (a)	30	0	52	3	23	0
June (b)	44	3	57	1	16	1
July (a)	38	9	69	3	24	2
July (b)	36	7	73	0	27	3
August (a)	43	9	74	1	29	0
August (b)	58	10	73	0	28	6
September (a)	23	11	63	1	28	5
October (a)	45	12	62	3	25	2
Totals	317	61	523	12	200	19

S.s. = *Scolytus scolytus*, *S.m.* = *Scolytus multistriatus*
(a) = sampled during the first half of the month.
(b) = sampled during the second half of the month.

pheromones, released by early colonists (Peacock *et al.*, 1971; Borden and King, 1977) cannot be excluded from the results' interpretation. During the second season the arrangement of test logs was changed slightly to give a mixed formation (i.e. 1 English, 1 Wheatley, 1 wych, etc., etc.) as three mixed rows of eighteen logs each. A bias favouring selection of English and Wheatley elm for colonisation still remains and since it now seems unlikely to be an artefact of experimental design, the existence of breeding preferences can be accepted.

Throughout this section results have shown wych elm logs to be least attractive for colonisation by *S. scolytus*. A number of interrelated factors are so far known to contribute to this effect. Within the diameter class of logs used throughout these experiments (5–20 cm) the thickness and degree of bark fissuring varies considerably between elm types. Wych elm logs of less than approximately 20 cm diameter often have smooth bark comprising a thin layer of inner bark or phloem and an even thinner layer of outer bark. Bark beetles are known generally to favour creviced areas for gallery initiation (Fisher, 1937; Beaver, 1967). Indeed, more recent work (Von Keyserlingk, 1979) has shown crevice searching to form an integral behavioural component for colonising *S. scolytus*.

When forced to breed in smooth wych elm logs in the laboratory, *S. scolytus* has been seen to enter around branch outgrowths or at a point of bark injury. In spite of this even those wych elm logs within the higher diameter range, with more fissured bark, still show fewer attacks than logs cut from English and Wheatley elm. It should also be noted that the mean diameter of wych elm logs was greater than that of English (i.e. wych 10.31, S.D. 2.28 cm, Wheatley, 9.75, S.D. 3.64 cm, English 8.71, S.D. 1.66 cm). Bark texture does not, therefore, account for the overall reduction in maternal gallery totals in wych elm.

Thickness of wych elm bark is also important in considering the effect of the fungus *Phomopsis oblonga* on beetle colonisation. The effect of *P. oblonga* on breeding and mortality has already been investigated (Webber, 1981) and its ability to affect broodwood utilisation is also known (O'Callaghan, 1982). As no direct mycological analysis was made during these experiments results obtained can only be taken as field observations. It has been noted, however, that wych elm logs showed the zone lines characteristic of *P. oblonga* before their corresponding appearance in English or Wheatley elm logs, and that in wych elm more of the bark area was affected than in the other elm types.

Thin barked logs were more readily colonised by *P. oblonga* and this has a concomitant influence on wych elm. It must also be assumed at this stage that thin bark (therefore the majority of wych elm logs) dries at a faster rate and that insufficient moisture could influence the number of beetles colonising such logs. So far the words "attack" and "colonise" have been used almost synonymously but in so far as identifying host preferences is concerned they may be quite different. As beetles neither attacked nor colonised wych elm logs infested with *P. oblonga* it seems that they are able to detect presence or absence of the fungus at an early stage and the role of moisture may be critical at this point.

The existence of an elm species preference for colonisation by *S. multistriatus* in order to breed is not easily demonstrated in the field. At first sight the data appears to show a distinct bias favouring selection of English elm (see Table 1). Certain considerations must be borne in mind when reviewing these data. Firstly, *S. multistriatus* emerges after *S. scolytus* therefore suitable broodwood may have already been infested. It has been shown that *S. multistriatus* favours logs within the narrow diameter range used in these experiments (see Figure 2) and any bias with regard to host type in test logs could also influence their relative attractiveness.

From data obtained over consecutive years we conclude that:

(i) *S. multistriatus* is attracted to logs already infested with *S. scolytus*.

(ii) Adult *S. multistriatus* are subsequently able to select suitable broodwood free of *S. scolytus*, in this instance there is a tendency towards logs in the narrow diameter range including wych elm logs free of *P. oblonga*.

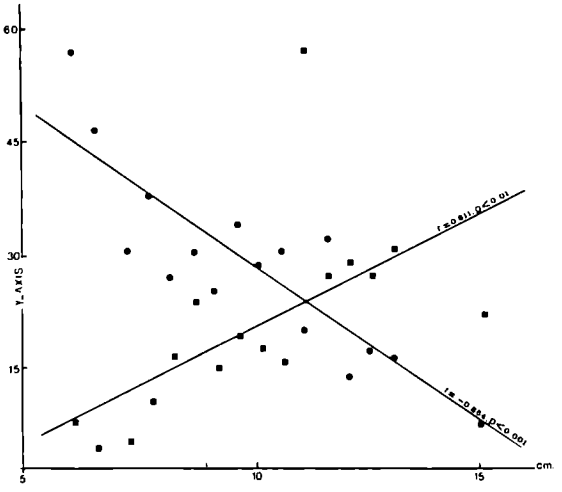

Figure 2. Maternal gallery density against log diameter in cm.
● *S. multistriatus* ■ *S. scolytus*

(iii) The low density attack of Wheatley elm in Table 1 (see also Figure 4b) is probably due to an existing high density population of *S. scolytus*.

(iv) In spite of the possible influence exerted by certain factors outlined above, *S. multistriatus* adults favour English elm for breeding when offered a choice using English, Wheatley and wych elm.

Before completing this particular section some mention of the effect of cut limbs must also be made. Byers *et al.*, (1980) have shown that cut elm limbs can attract more beetles than undamaged limbs. Since all of the logs used in these experiments had been removed from infected (DED) trees we must acknowledge that host volatiles may have attracted densities not always encountered in the field. Nevertheless, discussions have centred on comparative rather than numerical differences and therefore conclusions drawn are valid at this level.

6. *Area of attack*

Both *S. scolytus* and *S. multistriatus* show a significant bias favouring attack of the underside or shaded regions of horizontally positioned test logs, regardless of host species or variety. The bark surface of each sample disc has been considered as divisible into quadrants: A/B upper and C/D the lower surfaces with respect to horizontally positioned test logs. English elm logs located at Hyde, Manchester, for example, sustained a total of 254 attacks. Quadrant A contained 46 attacks, quadrant B, 43; quadrant C, 75 and quadrant D, 90: $\chi^2=$

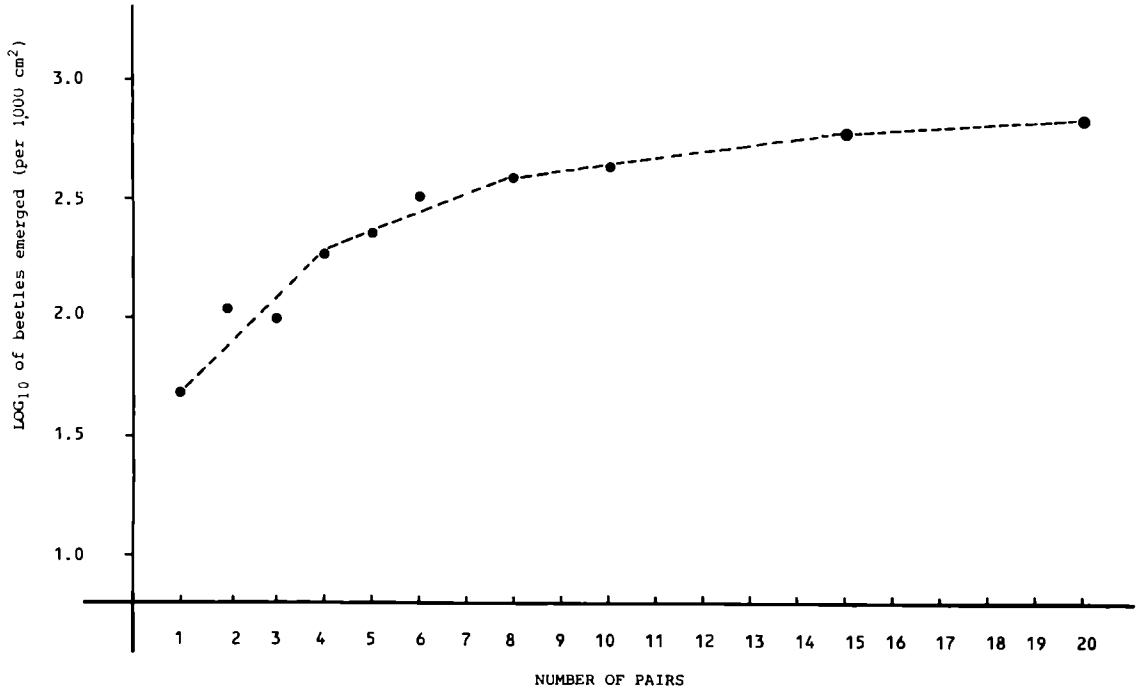

Figure 3. The relationship between number of progeny of *S. scolytus* emerged per 1,000 cm² of infested bark and the number of pairs of adult beetles introduced.

24.56, $p < 0.001$. Consistent differences were observed between upper and lower surfaces for each host type at each site. It is assumed that this preference is a result of two modes of behaviour:

(i) the cryptic habit giving rise to attack of creviced and/or shaded regions (Fransen, 1939);

(ii) the preference for selection of moist areas for breeding. The lower surfaces of horizontally position logs will retain moisture for a longer period and accumulate additional water due to 'run-off'.

Where high density attack occurs the pattern changes as the season advances and the number of colonists increases. In these instances the numbers colonising different portions of each log eventually become equal.

7. *Density of attack*

Preliminary investigations at Salford (Heaton and Sutton personal communication) have shown density levels in relation to brood output to peak at between 8 and 10 maternals per 1,000 cm² or between 100 and 125 cm² per *S. scolytus* gallery (see Figure 3). This estimate agrees well with both the data of Webber (1981) where the optimum area requirement per *S. scolytus* gallery was set at 100 cm², and the range of densities recorded from field sampling. Figure 4(a), (b), (c) depicts just one series of data for densities within logs of each elm type situated at the Cheshire site. Density of *S. scolytus* galleries is seen to increase with increasing log diameter using English elm. In Wheatley elm, where log diameter range extended to 20 cm, there is a levelling out with density estimates not exceeding 24 galleries per 1,000 cm². Density of *S. scolytus* in wych elm remains low throughout the diameter range. Estimates for *S. multistriatus* do not attain those of *S. scolytus* in logs located in northern England, (maximum density = 8 galleries/1,000 cm² in logs 7.0 cm diameter) but exceed those of *S. scolytus* (54.8 galleries/1,000 cm² in logs 6.5 cm diameter) in narrow diameter English elm logs located in southern England (Devon).

Considering these densities it is likely that Wheatley elm located at Northwich, Cheshire (see Table 1) contained gallery totals exceeding those capable of yielding maximum brood output. Those for English elm are within the maximum range and those for wych elm are low. This would seem to indicate that for certain hosts in northern England intraspecific competition is a major mortality factor restricting potential brood output. This would be especially relevant for the south of England dominated by English elm, one of the most favoured host species.

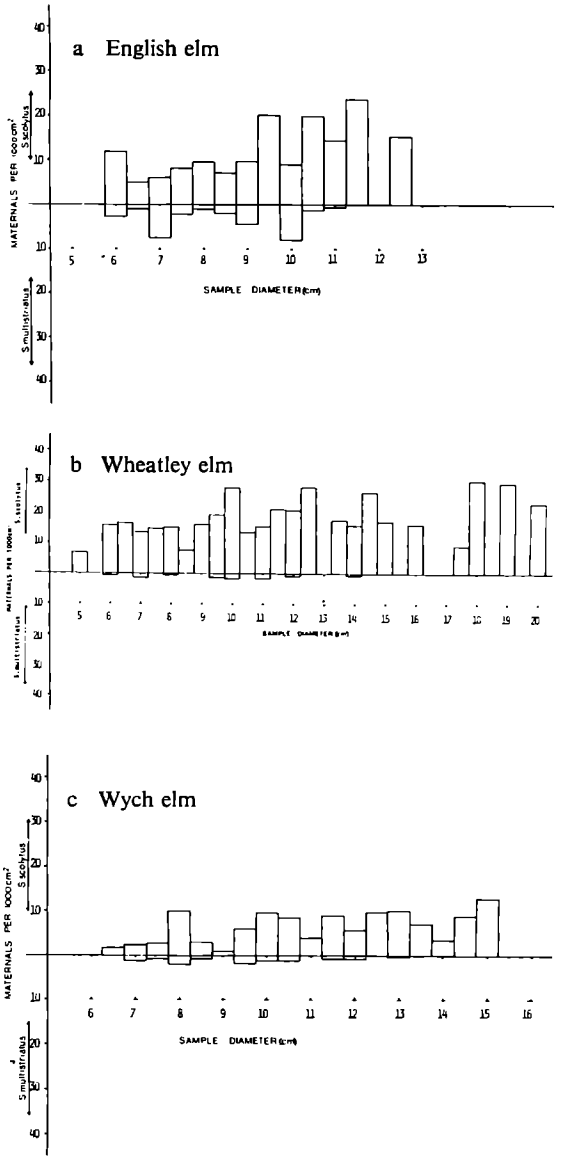

Figure 4. Maternal gallery density (per 1,000 cm²) against test log diameter (cm).
(Logs situated at Northwich, Cheshire)

8. *Beetle productivity*

Eggs of both *S. scolytus* and *S. multistriatus* are deposited regularly down each side of the maternal gallery as it is cut. It is not surprising, therefore, to find that a significant positive relationship exists between the length of gallery cut and the number of eggs deposited (*S. scolytus* $r = 0.912$, $p < 0.001$; *S. multistriatus* $r = 0.813$, $p < 0.001$; see Figure 5). Of greater interest is the realisation that the length

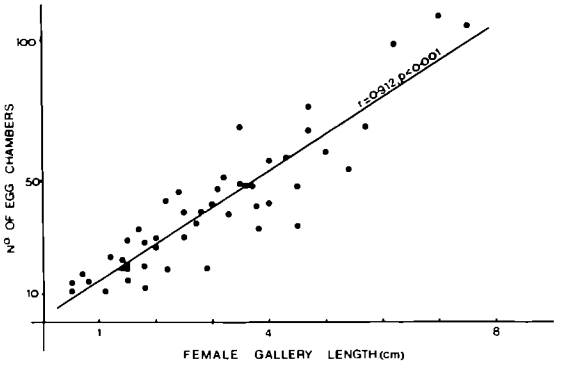

Figure 5. S. scolytus: gallery length (cm) against number of egg chambers.

of gallery cut and therefore the number of eggs deposited varies latitudinally throughout Britain. Table 2 shows the results of analyses carried out on 1979 and 1980 data for sites in Scotland, northern and southern England.

Vector performance can be seen to decline as distribution extends northwards. Although there is a significant difference between data sets for Scotland versus northern England and between northern England and southern England there is no difference between northern sites with respect to elm type. A similar trend in gallery length reduction also exists for *S. multistriatus* between northern and southern England but these data are not significantly different ($p > 0.05$).

Once again the presence of *P. oblonga* confuses the issue in terms of separating the effect of latitude from the possible effect of host identity. Since logs cut from wych elm were infested by *P. oblonga* much earlier in the season in northern England it may well be that beetles are less inclined to engage in gallery construction and egg laying for prolonged periods. The role of log sample diameter must also be borne in mind for it is possible that climatic influences are more apparent in narrow diameter logs than in major bole sections. Analysis of infested trunk sections may yield a different pattern north versus south.

Beetle productivity relies primarily of course on whether each female constructs one or more than one gallery. Samples of English elm cut during August and September from logs situated in Devon contained a total of 323 complete* galleries. Adult *S. scolytus* females were found (all dead) in 50 of the 127 galleries (39.4 per cent). Adult *S. multistriatus* females (all dead) were found in 129 out of 196 galleries (65.8 per cent). Only one male of each species was found in the galleries which could suggest polygamy for males but not exclude the possibility of females, particularly of *S. scolytus*, constructing more than one gallery. On no occasion have live adults been found in any portion of infested bark or wood during the winter months.

9. *Infestation of outer sapwood by late instar larvae*

It has been reported previously (Fisher, 1937; Beaver, 1967; Kirby, 1980) that a proportion of *S. scolytus* larvae, having reached fourth and fifth instar, will excavate cells in the outer sapwood. This behaviour has not been noted for *S. multistriatus* and only for *S. scolytus* larvae destined to overwinter. Beaver (1967) referred to these as pupal cells but, since it is the larva and not the pupa that may occupy the cell for up to nine months, they may be more accurately described as larval cells. Only during the final stages of metamorphosis does the larva within the wood cell pupate and finally become an imago.

Although *S. multistriatus* larvae pupate in the bark layers large numbers of wood cells have been recorded in logs infested by *S. laevis*. Lekander *et al.* (1977) considered pupation in the wood to be an integral part of the life history of *S. laevis*. From the limited number of vacated elm bolts inspected it would seem that a high proportion of *S. laevis* larvae cut wood cells (see Figure 6) to an appreciable depth under British conditions.

* 'Complete' meaning the whole maternal gallery was present in the sample thus allowing positive location of adult beetles if present.

Table 2. Regression analysis showing changes in female gallery length and number of eggs laid by *S. scolytus* in different parts of Britain.

Site	Scotland	Cheshire, England			Greater Manchester, England			Devon, England
Host elm species	Wych	English	Wheatley	Wych	English	Wheatley	Wych	English
Mean length (cm)	2.34	2.97	3.30	1.75	2.72	2.26	2.08	4.37
Standard deviation	1.40	1.64	1.79	0.92	1.19	1.09	0.87	2.22
Mean number of egg chambers	32.17	40.38	44.64	24.24	41.06	33.96	25.54	60.2
Standard deviation	20.33	23.31	24.06	12.25	21.47	17.48	12.54	29.05
Slope of y on x	13.04	12.94	12.10	11.50	15.20	13.20	11.42	9.82

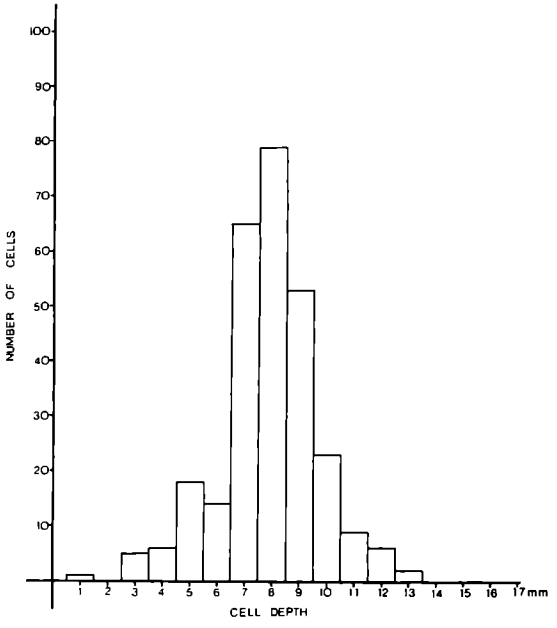

Figure 6. Scolytus laevis: Depths of pupal cells in outer sapwood of wych elm.

The number of *S. scolytus* larvae behaving in this manner varies with latitude but little information is so far available on those factors affecting wood cell formation. Tables 3 and 4 show the preliminary analysis of records of *S. scolytus* infesting logs at different locations.

By comparing these data we may construct, albeit tenuously, a probability list for the importance of some of the factors thought to influence wood cell formation by *S. scolytus*. For northern England cell depth is greater in English than in Wheatley, and greater in Wheatley than in wych elm samples. Comparing northern and southern England (English elm only) cell depth is greater north versus south as is the number of cells. Comparing Scotland and northern England (wych elm only) cell depth is greater in Scotland than England as is the number of cells. Looking at the number of cells cut in northern England during 1979 and 1980 it is evident that more cells were cut during 1979 (a particularly harsh winter and delayed spring) than in 1980.

Ambient temperature seems to be the most influential of environmental factors, therefore there is some justification in implicating bark thickness and bark type, creviced bark possibly being a better insulative layer than smooth bark. However, cell depth is greater in English elm samples in spite of the fact that this species possesses the thickest and most deeply creviced bark. Barson (1974) has shown that larvae occupying the dry outer layers of the bark, the spent layers of inner phloem and the sapwood are more likely to survive extreme temperatures (—15°C to —20°C) than larvae situated

Table 3. Analysis of 1979 field season's results for wood cell formation by overwintering *S. scolytus* larvae.

Site	Cheshire*	Greater Manchester			Devon
Host elm species	English	English	Wheatley	Wych	English
Number of Cells	28	7	73	21	44
Number of Galleries	38	34	22	14	447
Mean cell depth (mm)	5.25	4.71	6.51	5.23	5.36
Standard deviation	1.79	1.25	3.21	1.84	1.97
Standard error	0.33	0.47	0.37	0.40	0.29
Cells per 100 galleries	73.7	20.6	331.8	150.0	0.84

*The 1979 Cheshire site used English elm only.

Table 4. Analysis of 1980 field season's results for wood cell formation by overwintering *S. scolytus* larvae.

Site	Cheshire			Greater Manchester			Edinburgh
Host elm species	English	Wheatley	Wych	English	Wheatley	Wych	Wych
Number of Cells	35	102	0	54	78	3	31
Number of Galleries	68	113	53	67	45	18	17
Mean cell depth (mm)	7.14	5.57	–	7.02	5.18	4.33	6.54
Standard deviation	2.46	2.32	–	2.88	2.54	1.15	2.51
Standard error	0.41	0.22	–	0.39	0.28	0.66	0.45
Cells per 100 galleries	51.4	90.2	–	80.5	173.3	16.6	182.9

in or near damp portions of phloem. The role of bark in relation to temperature is, therefore, questionable and greater knowledge of the physical properties of bark is required.

Fransen (1939) records finding more wood cells in narrow diameter logs than in thicker sections, but also reported increased wood cell formation where high larval densities occurred. In our experiments the highest *S. scolytus* larval densities occurred in English elm in southern England, but these logs contained fewer wood cells than logs containing lower larval densities in northern England. Looking at high density attack in northern logs, however, we observed an increased tendency for wood cell formation. We can only conclude at this stage that if larval density is a key factor it is somehow synergised by more adverse climate, or in the case of southern England, overridden by warmer weather. Michalski (1973) suggested that humidity affects wood cell formation and this in turn would be influenced by log diameter, bark thickness and degree of fissuring.

Natural mortality

The role of *P. oblonga* in elm bark beetle mortality has already been discussed. The only other predators seen to seriously affect large numbers of beetle larvae were birds (woodpeckers) and hymenopteran wasps. Woodpecker attack was not a problem in so far as test logs were concerned in most northern sites. In Cheshire, however, the bark of almost all test logs was entirely stripped during late summer and autumn. These attacks caused major losses of larvae at fourth and fifth instar and also during pupation.

The only insect parasite found to cause large scale losses on a localised scale was the Brachonid, *Coeloides scolyticida* (Wesmael). This species attacked very early stage larvae but subsequent death only occurred with the larvae at a late stage of development. Fransen (1939) also found *Coeloides* to be the most important natural predator in Holland and Beaver (1966) found it to be responsible for high larval mortality amongst pre-epidemic scolytid populations in southern England. Adults of *C. scolyticida* were observed on the wing and ovipositing during warm, sunny days with air temperatures above 18°C. Only one generation could be definitely assigned to the 1979 and 1980 seasons although this species was not studied in great detail.

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PAPER 8

The effect of climatic factors on the dispersal of elm bark beetles

C. P. FAIRHURST* and C. J. KING†
*Department of Biology, University of Salford, Salford, M5 4WT, U.K.
†Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH, U.K.

Introduction

The bark beetles, *Scolytus scolytus* (F.) and *S. multistriatus* (Marsham) are known as the main natural vectors of Dutch elm disease (DED) in Britain, and it is obvious that the dispersal flights of these insects will be affected by climate. Water (1982) co-ordinated field catches, using pheromone baits, at 20 sites throughout the Netherlands and concluded that 84 per cent of the beetles were trapped on days when the maximum temperature exceeded 21°C. This agrees with the work of Fransen (1939), who found that *S. scolytus* emerged at 17.5°C and would fly at 20°C with an optimum at 25°C. The studies of Von Keyserlingk (1980) indicated emergence and flight thresholds at 16° and 20°C respectively and that a period of pre-heating was necessary. For *S. multistriatus*, Norris (1965) found emergence at 15–18°C and flight at 22–23°C, whilst Fransen (1939) and Bartels and Lanier (1974) considered that the threshold for the latter factor was 20°C. There are indications that above these temperatures, wind speed, barometric pressure and rainfall may affect the total number of beetles in flight or reaching the pheromone traps (Von Keyserlingk, 1980; Lanier and Burns, 1978). Temperatures over 30°C may cause reversal of the positive photic response demonstrated by Meyer and Norris (1973) and this may explain that fact that in areas of America where the beetles fly daily between April and October, the major activity is in the mornings (Svihra, 1982).

In Britain, the rate of spread of the epidemic caused by the aggressive strain *Ceratocystis* (*Ophiostoma*) *ulmi* (Buisman) Nannf., is less in the north of England than in the south (Kirby, 1980; Kirby and Fairhurst, 1981a, b; Webber, 1981). This trend also applied to the epidemic caused by the non-aggressive strain starting in the 1930s (Peace, 1960). The question remains, whether this feature was due to a change in the species composition and abundance of *Ulmus* species (Gibbs and Howell, 1972, 1974; Burdekin, 1981), the effect of latitude on beetle productivity (Kirby, 1980; Kirby and Fairhurst, 1981a, b), the increased presence of *Phomopsis oblonga* (Desm.) Trav., known to interrupt galleries (Webber, 1981), or the effect of climate in restricting bark beetle dispersal. The answer is probably a combination of these factors plus an unpredictable element introduced by the illegal importation of infested timber (Redfern, 1977; Bliss, 1981).

This paper attempts to assess in broad terms, the number, character and distribution of potential flight days, defined as days when the maximum temperature equals or exceeds 22°C.

Temperature and flight days

The numbers of days exceeding 21°C is not immediately available from published meterological information. Therefore it was decided to take four weather stations in the north-west of Britain, representing different geographical characteristics:

Sheltered urban — Manchester Weather Centre.
Sheltered forest — Delamere Forest, Cheshire.
Sheltered coastal — Colwyn Bay, Clwyd.
Exposed coastal — R.A.F. Valley, Gwynned.

Initial studies confirmed that the best flight day correlation existed with the average daily maximum temperature. Not only were these regressions significant at the $p<0.001$ level for the months of May, June, July, August and September, but also there was no significant difference between sites. These relationships are shown in Figure 1, and it is important to notice the differences in slope of the plotted data between the months. In September and May, for example, relatively low average temperatures may give potential flight days, but the maximum number of such days is not great. In contrast, July and August, can be relatively warm, leading to rapid larval development, but still not provide flight days. Obviously, the maximum number of flight days are possible in June, July and August. Talks with the Manchester Weather Centre indicate that these relationships hold reasonably true for the rest of Britain.

Maps of average maximum temperatures (Meteorological Office, 1975) were not used as the values

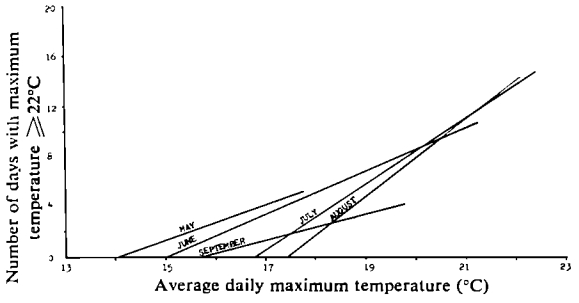

Figure 1. Relationship of number of days ⩾22°C with average daily maximum temperatures. Original data from the Manchester Weather Centre covering the period 1961–1981. Correlation coefficients (r); May: 0.675, June: 0.913, July: 0.967, August: 0.917, September: 0.653, n=21.

are reduced to sea level equivalent, and less weight is given to urban areas. Therefore the tables of actual values from 233 meteorological stations in Great Britain, the Isle of Man and the Channel Islands were used for the period 1941 to 1970 (Meteorological Office, 1976). The number of days ⩾22°C were read off the regression lines and are summarized in Figure 2 for individual months and for the annual totals. The general trends with latitude, altitude, exposure and proximity to the sea can be noticed.

The errors inherent in these estimates are that:

(a) there is some residual error from the regression,

(b) some counties are not covered by weather stations,

(c) the time period covered by the base data does not cover the main period of the current epidemic,
and

(d) each site is affected by local factors.

This last feature is worth further consideration. Aspect, shelter, water bodies and land use may all affect temperature, sunshine, wind speed and direction, humidity and rainfall. For example, the centre of an urban area such as London may have a maximum temperature 3°C higher and a minimum temperature 6°C higher than suburbs (Chandler, 1965) and the average wind speed may be 25 per cent lower. On a larger scale, the shelter provided by ranges of hills can make appreciable differences to temperature. A moist wind has an adiabatic cooling lapse rate of 0.5 to 0.6°C per 100 metres altitude. Having lost moisture over upland areas, the dry, descending wind gains almost 1°C per 100 metres. This phenomenon, known as the Fohn effect, goes some way to explain the relatively high temperatures in the Cheshire bowl and other areas in the lee of major hill areas.

It is difficult to judge the potential flight days on larger islands from one weather station, but it is interesting to note that Guernsey Airport (104 m altitude) has an estimated 15 beetle flight days per year, the same value as Dundee (45 m). St. Helier on Jersey (9 m) has a similar 33–34 days to Alice Holt Lodge (115 m) and Winnington in Cheshire (20 m). Contrasts can also be found, for example in Tyne and Wear, where Tynemouth (29 m) has an estimated two days, while Chopwellwood (136 m) some 20 miles inland has over 16 days. The highest estimates are, as expected, in the Thames Valley, reaching a maximum of over 47 days in Greater London.

The known northern limit of DED, and by implication *S. scolytus*, correlates with the line representing 10 days. Although information on the northern range of *S. multistriatus* is at present scanty, the 20 day line may be appropriate.

Variations between years

The estimates presented above are based on averages over many years and annual variations can be considerable. Figure 3 represents the annual total flight days at the Manchester Weather Centre as compared to the exposed site at R.A.F. Valley.

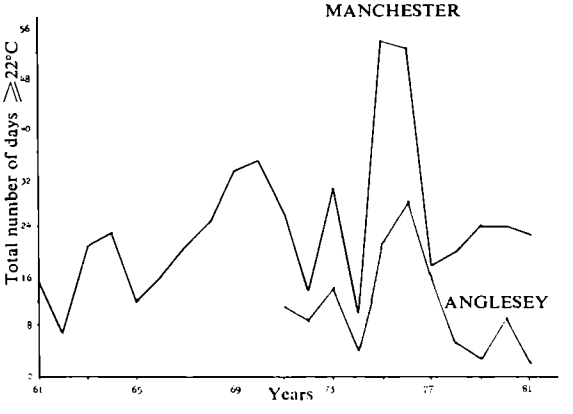

Figure 3. Distribution of days ⩾22°C with year.

In some years the potential for major dispersal is very limited. It is, however the coincidence of two hot summers such as 1975 and 1976, which correlates with the major northern (Redfern, 1977; Crowson, 1976) and western (Jones, 1981) range expansion of DED.

Figure 2. Maps summarizing the number of days $\geqslant$22°C, per summer month, and annual totals.

Table 1. Distribution of number of days ⩾22°C by months over the period of the current epidemic. Data taken from the Manchester Weather Centre.

Year	May	June	July	August	September	Other months	Total
1968	2	9	3 (1)	9	2	0	25
1969	0	8	13	9	1	2	33
1970	3	14	3	12	3	0	35
1971	0	0	15	4	6	1	26
1972	0	0	9	3	2	0	14
1973	2	9	7	9	3	0	30
1974	0	5	2	3	0	0	10
1975	0	11	14	18 (14)	1	0	54
1976	2	13 (2)	17 (7)	21	0	0	53
1977	2	4	7	4	1	0	18
1978	6	5	5	2	0	2	20
1979	3	6	9	5	0	1	24
1980	7	3	6	7	1	0	24
1981	1	0	5	10	7	0	23

Figures in brackets refer to number of days ⩾30°C.

It is also of interest to note that the major northward spread of the earlier non-aggressive epidemic coincided with the considerably warmer summers of the 1930s, 1940s and early 1950s (Barry and Chorley, 1976) which affected the ranges of many other flying insects (Kaisela, 1962). A return to a colder period could have lead to stabilisation of the DED front (Peace, 1960).

The distribution of flight days within each year must also be important. In Manchester, the estimated 35 flight days in 1970 were distributed mainly in June and August with only three in July (Table 1). This pattern means that the overwintering and the summer generations of *S. scolytus* are both able to disperse and 1970 is thought to be a year when major spread of the disease was noted in the Midlands and East Anglia. However, there were no flight days in May or June in either 1971 or 1972, so that any adults emerging from the overwintering generation of *S. scolytus* would be restricted in dispersal ability. These years showed reduced spread of the disease (Jones, 1981). Years with the main flight days occurring in July, may in fact favour *S. multistriatus*, able to exist with mainly one generation per year.

A series of flight days usually occurs when an anti-cyclone stabilises over the continent of Europe, leading to a persistent ridge of high pressure across Britain or a depression to the north of Scotland. This leads to stable barometric pressure and a light south-easterly air-stream.

Normally, in June, July and August, a western coastal site will have winds in excess of 5 m/sec., known to be incompatible with flight (Keyserlingk, 1980) and approximately half the days have winds from the south to west quadrant. On days with a temperature equal or exceeding 22°C, easterly winds are predominant (Figure 4) and most of the wind speeds are less than 4 metres per second. Similar patterns have been found at other sites.

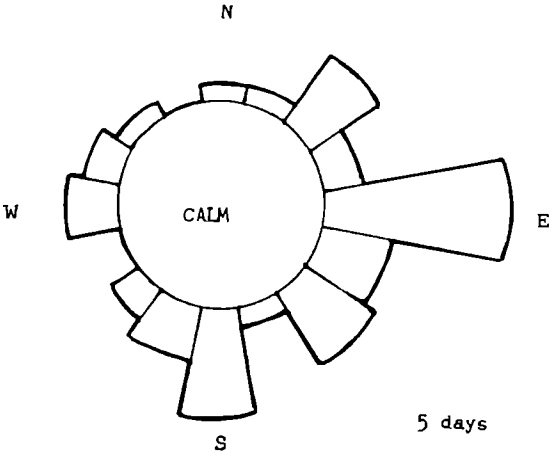

Figure 4. Wind rose for days ⩾22°C at R.A.F. Valley, Anglesey for 1971–1981.

Micro-climate

Regional variation may set the trends, but for individual trees and beetles, micro-climate must be considered. Slope, aspect, shading, shelter and surface albedo will greatly affect temperatures. Gajzago (1973) quotes an example where the air temperature is 17°C and the ground temperatures underneath park trees on the shaded and sunny sides were 14° and 27°C respectively. Shelter belts are well known for the amelioration of wind speeds and these values are halved below crown height in

deciduous woodland (Geiger, 1967), being reduced normally to less than 1 metre per second at half crown height. Geiger also quotes a temperature profile in deciduous forest where the level was 23.5°C above the crown, 27°C in the crown, and 21.5°, 20.5° and 18.5°C at 19, 11 and 3 metres respectively above the forest floor. Thus, the potential for beetle dispersal may be reduced in woodland areas.

These temperature differences also effect emergence and a good example is given by Daterman *et al.* (1965) who measured air and inner bark temperatures in shaded and clearcut conifer plots (Figure 5). These differences lead to a delay of 40 days in the emergence of *Dendroctonus pseudotsugae*, Hopkins.

The effect of temperature on larval development

In addition to the reduction in the length of the maternal gallery and number of larvae, noted by Kirby and Fairhurst (1981) for *S. scolytus*, the rate of development will be dependant on meteorological circumstances although not necessarily air temperature. This may have resulted in an additional number of generations in, for example the 1975 and 1976 summers. Fransen (1939) noted four generations in *S. scolytus* and two in *S. multistriatus* in the warm summers of 1930 and 1932.

Such differences in speed of development will depend on accumulated temperature. Maps of this parameter contrast with Figure 7 in that Atlantic influences lead to a more equable climate in the south-west and coastal areas. The influence of winter cold will also be less noticeable in these areas, although freezing temperatures are unlikely to cause significant larval mortality in Britain. Barson (1974) indicated that the Lt_{50} for *S. scolytus* larvae is approximately —20°C. Wallace and Beard (1943) and Thomas (1971) show that *S. multistriatus* is not killed by such temperatures, which are unlikely to be reached in elm timber (Sakai, 1966).

Discussion

Beaver (1979) considered the transient nature of dead trees and the consequent necessity for dispersive and colonizing abilities of the bark beetle species. The population dynamics of bark beetles are often characterised by dramatic changes in abundance. Raffa and Berryman (1980) point out that bark beetle habitats can be considered at two levels: the individual host tree, and the distribution of these trees. The selection of suitable breeding material therefore causes divergent selective behaviour patterns.

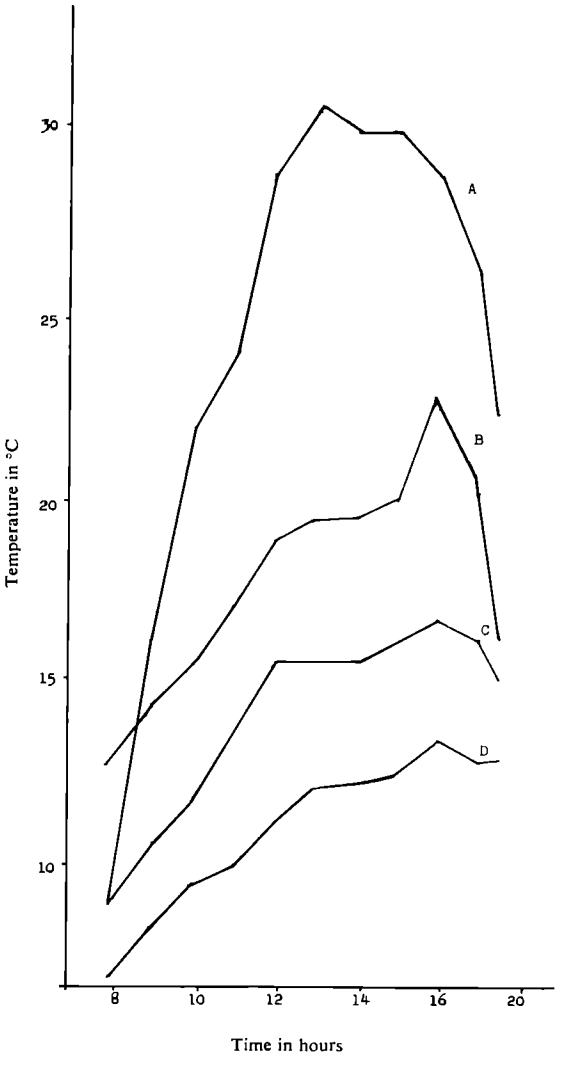

Figure 5. Comparison of air and bark temperatures in clearcut and shaded areas, May 13, 1963, with time of day.
A = inner bark temperature, clearcut plot.
B = air temperature, clearcut plot.
C = air temperature, shaded plot.
D = inner bark temperature, shaded plot.
Data taken from Daterman, Rudinsky and Nagel (1965).

The work of Beaver (1966) and Schröder (1974) indicate larval mortality of some 80 per cent. Taking the number of larval mines in the north of England to be approximately 40 per *S. scolytus* maternal gallery, approximately four daughter females will emerge per parental female. Bearing in mind adult mortality, the barriers provided by topography, the lower density of elms in the north and factors

such as *Phomopsis* preventing successful colonisation, the host-seeking behavioural responses must be highly developed. It could be hypothesised that primary host attraction should exist, but this has not been recorded for *S. scolytus*.

At flight temperatures, the beetles lose water quickly and death may result in three days (Keyserlingk, 1980). Fransen (1939) also observed that the life expectancy of males was 2 to 14 days. Therefore, the insects must be sufficiently aware of environmental factors to utilize the optimum dispersal periods. Should these conditions not be reached, the beetles will remain on or near the tree where they emerged.

It is likely that Peace (1960) was right in his assumption that *S. scolytus* beetles were rare or absent in Scotland prior to the 1930s epidemic. The introduction of DED into Great Britain at that time and the consequent availability of host material coincided with a series of warm summers which enhanced the spread of the disease. A decline occurred in the subsequent colder period, but the warmer years in the 1970s saw the spread of the aggressive strain of *C. ulmi* in the north.

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PAPER 9

Host feeding preference of *Scolytus scolytus*

J. F. WEBBER* and S. G. KIRBY†

*Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH, U.K.

†Department of Biology, University of Salford, Salford, M5 4WT, U.K.

Introduction

In Britain there are three principal elm species although many more varieties and hybrids exist (Clapham *et al.*, 1962). The main species are English elm, *Ulmus procera* Salisb.; wych elm, *U. glabra* Huds. and the smoothleaved elm, *U. carpinifolia* Gleditsch. The English elm, introduced into this country during the late Roman period (Richens, 1976), is the dominant species in the south of England making up a large proportion of our hedgerow trees. The smoothleaved elm is also an introduced species but found mainly in East Anglia and the East Midlands. The only native species is the wych elm, often found as a woodland tree, and the major elm species in northern England, Wales and Scotland (Gibbs, 1974).

The early surveys of Dutch elm disease indicated that all three species were susceptible to the aggressive strain of *Ceratocystis ulmi* (Buis.) Moreau, but apparently to varying degrees (Gibbs and Howell, 1972, 1974). Comparisons showed that the incidence of disease in English elm was significantly higher than in both wych and smoothleaved elm, and this difference was ascribed to English elm being the most susceptible of the three species. However, when Brasier (1977) inoculated young English and wych elm with isolates of *C. ulmi* he found that wych elm proved to be markedly more susceptible to the fungus than English elm. He suggested therefore, that the better field performance of wych elm in the present Dutch elm disease epidemic was probably due to its relative attractiveness to the beetle rather than to its intrinsic resistance to the fungus. With this in mind we decided to examine the preferences of the larger elm bark beetle, *Scolytus scolytus* F., when presented with both English and wych elm as feeding material.

Testing for feeding preference

All beetles used in this experimental work were taken from a southern population of *S. scolytus*, having emerged from brood material collected from the south of England. Four-year-old trees of English elm and wych elm, *c.* 160–200 cm in height and growing in pots, were used as the host material. The number of potential feeding positions on each tree was estimated by counting the total number of twig crotches formed at the junction of old twig growth (old being at least the previous season's growth), these having been observed to be most commonly used by *S. scolytus* for feeding in the field. On this basis it was ensured that the same number of potential feeding positions was supplied for both elm species.

Six trees of each species were selected and arranged in a plunge bed in two rows of alternate English and wych elm (Figure 1a). The whole plunge bed was

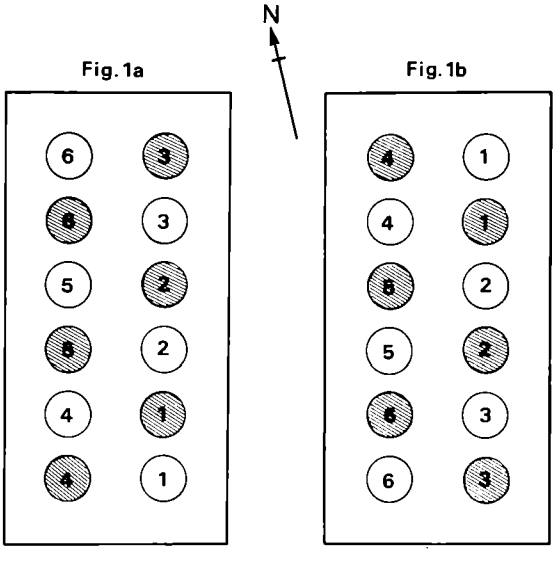

Figure 1. Figure 1a illustrates the layout of the trees in the plunge bed during days 1–7. Figure 1b illustrates tree positions during days 8–24. Open circles (O) correspond to *U. procera* (English elm); cross-hatched circles (⊗) correspond to *U. glabra* (wych elm). The summed number of potential feeding positions on all the trees of *U. procera* amounted to 119, and 118 on *U. glabra*.

then enclosed in a 2 m high rectangular nylon net tent. Except during rainy days, equal numbers of male and female beetles were introduced daily into this enclosure over a two week period. In addition, halfway through the experiment the order of the trees in each row was reversed (Figure 1b) to counteract any positional effects which might have encouraged beetles to feed on certain trees. The number of fresh feeding crotches cut on each tree was usually assessed on a daily basis for the two weeks of the experiment and a final count was also made on the twenty-fourth day after the start of the experiment.

Results and conclusions

It was apparent during the first few days after the onset of the experiment that beetle feeding was more frequent on English elm than on wych elm (Figure 2). The mean number of feeding grooves per tree cut on wych elm never exceeded nor even approached the mean number recorded on English elm and statistical analysis confirmed this was a significant difference ($P < 0.05$) from the ninth day onwards. However, there was considerable variation in the number of feeding grooves cut on individual trees within a single species (Table 1) and trees selected by beetles for feeding in the initial stages of the experiment (e.g. *U. procera* trees 1 and 2) tended to be the ones most heavily fed on for the remainder. On some English elm feeding was so intensive that the number of feeding grooves cut exceeded the estimated number of potential feeding positions. As expected the most frequent position for feeding was in the junctions of 'old on old' twigs, but beetles were also observed feeding at the twig bases of the current year's growth and, more occasionally, at the base of leaf petioles. In English elm feeding even occurred on the corky wings of bark present on some twigs, and on the rougher bark of the trunk and major branches.

It is clear from these results that beetles derived from a southern population of *S. scolytus* prefer to feed on English elm. At present it is only possible to speculate how and why this preference occurs. Selection of elm material for feeding or breeding in preference to other tree genera is believed to be made by *Scolytus* species on the basis of host volatiles and host form/habit characteristics (Baker and Norris, 1968; Choudhury, 1976), therefore beetles may be able to distinguish between the various elm species in the same way. However, it may be that selection between elm species is governed not by response to species specific volatiles or habit, but made largely on the grounds of preference for certain bark textures. The early work of Fransen (1939) established that beetles tended to initiate feeding in the neighbourhood of old scars or other roughened and protected places. Both bark and twig crotches of English elm are invariably

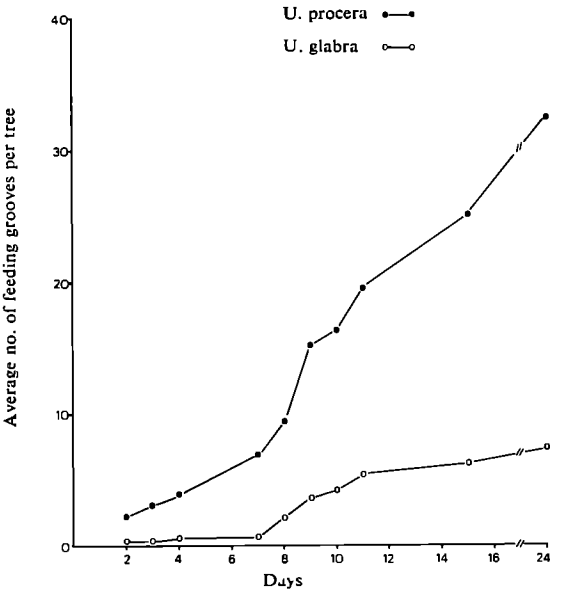

Figure 2. Comparison of beetle feeding on *U. glabra* and *U. procera*.

Table 1. Number of feeding grooves cut by *S. scolytus* on *U. procera* and *U. glabra*.

	Ulmus procera							*Ulmus glabra*							
	1	2	3	4	5	6	$\bar{x}$	1	2	3	4	5	6	$\bar{y}$	
DAY 4	5	12	0	3	2	2	4.0	0	0	0	0	2	2	0.7	NS
DAY 8	9	28	0	13	5	2	9.5	7	0	0	1	3	2	2.2	NS
DAY 11	26	48	6	17	12	9	19.7	16	0	10	1	4	2	5.5	$P < 0.05$
DAY 15	30	61	8	21	20	11	25.2	18	0	12	1	5	2	6.3	$P < 0.05$
DAY 24	31	88	10	23	31	12	32.5	19	1	16	2	5	2	7.5	$P < 0.05$

The data show the cumulative totals of feeding grooves cut on six trees of *U. procera* (indicated by numbers 1–6) and six trees of *U. glabra* (again indicated by numbers 1–6) at intervals over a 24 day period.
For statistical analysis the data were transformed to square roots.

roughened or wrinkled, whereas the bark of wych elm tends to be much smoother. It is suggested therefore, that the initial preference of beetles for feeding on English elm is made because the numerous irregularities in the bark stimulate feeding.

Once feeding has been initiated on certain trees the situation is likely to become more complex and preference for one species over the other may then be reinforced by other factors. It is possible that when a number of feeding grooves are present on a tree, there is a release (or increased release) of host volatiles making the tree more attractive to beetles. Indeed, pruning wounds made on various species of healthy elm, including English and American (*U. americana*), have been found to significantly increase the number of scolytids attracted to the trees (Byers *et al.*, 1980; Landwehr *et al.*, 1981), and it is thought that this is due to the release of volatile host attractants such as α–cubebene. In addition, some beetles may produce their own pheromone attractants causing aggregation on certain trees. Evidence of this was indicated by the courtship behaviour and copulation which was occasionally observed in the feeding grooves of trees with heavy activity of beetle feeding. Such courtship behaviour in twig crotches has also been observed in *S. multistriatus* (Svihra and Clark, 1980).

As yet we do not know whether other populations of *S. scolytus* will react in the same way as did the population studied here. It will be of particular interest to know if *S. scolytus* from northern Britain, where wych elm is the predominant elm, shows a preference for wych elm over English.

To conclude, it seems likely that the better field performance of wych elm in the early 1970s was due to its rejection by feeding *S. scolytus* beetles, the lack of feeding attacks resulting in a low rate of infection by *C. ulmi*. In contrast, the heavier feeding on English elm resulted in a higher disease incidence despite the greater resistance of this species to the disease. If it proves to be true that the wych elm is unattractive to feeding beetles because of the smooth bark on young twigs and twig crotches, this could well be a characteristic that should be selected when breeding for elms resistant to Dutch elm disease.

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PAPER 10

Scolytid pheromone research in West Germany

D. KLIMETZEK and H.-P. KOPP
Institute of Forest Zoology, University of Freiburg im Breisgau, Federal Republic of Germany.

Introduction

Elm bark beetles of the genus *Scolytus* and *Pteleobius* are important vectors of Dutch elm disease (DED) in Europe, causing serious damage to forests and trees in urban areas. Control of DED can be directed against the pathogen *Ceratocystis ulmi* (Buis.) Mor. and/or its vectors. In both cases, knowledge of time and intensity of beetle flight is an advantage. Investigations at the Forstzoologisches Institut, Freiburg (FZI) were primarily concerned with the use of pheromone traps in monitoring and surveillance of elm bark beetles.

Evaluation of synthetic baits for Scolytus species

Earlier investigations in the Upper Rhine Valley indicated a preference for the δ-isomer of multistriatin (Gerken *et al.*, 1978) by endemic *S. multistriatus* (Marsh.), whereas American populations produce, and are attracted to, α-multistriatin (Gore *et al.*, 1977). Also, α-multistriatin seemed to inhibit response by *S. scolytus* (F.) (Gerken and Grüne, 1978; Blight *et al.*, 1980a). We therefore tested the field response to these multistriatin isomers by both *Scolytus* species in order to obtain information as to the most effective bait mixture, as well as to see if a single pheromone mixture could be used for both beetle species. Test compounds were commercial α-multistriatin (Chemical Samples, Columbus, Ohio) and (-)-δ-multistriatin of high chemical and optical purity as provided by Dr. W. Helbig, University of Tübingen (Hoffmann and Helbig, 1981). Both multistriatins were diluted to comparable standard concentrations with heptan (a, d); a further 10-fold dilution (a^{-1}, d^{-1}) was also applied. Baits used were racemic 4-methyl-3-heptanol plus cubeboil with or without addition of the test compounds. The pheromone components emanated separately from glass capillaries; traps without bait served as control. All treatments were rotated after each control in order to reduce the influence of trap positions.

Combination of 4-methyl-3-heptanol and cubeboil with the standard concentration of commercial α-multistriatin caught significantly more *S. multistriatus* in window traps than the combination with (-)-δ-multistriatin, although differences were not consistent at the 10-fold lower concentrations (Table 1). Preference of European populations of *S. multistriatus* for the α-isomer of multistriatin was also confirmed by using ventilated landing traps ("sleeve olfactometers") (Klimetzek *et al.*, 1981). These results agree with American experiences and field tests performed in the UK and in Switzerland (Lanier *et al.*, 1977; Blight *et al.*, 1980b; Angst, 1981). Earlier divergent results may have resulted from different concentrations of test chemicals used and dosage/response-reactions of the beetles (c.f. Grant and Lanier, 1982; Angst *et al.*, 1982).

No consistent difference was found between multistriatin treatments for *S. scolytus* (Table 1). There was also no preference for 4-methyl-3-heptanol plus cubeboil alone, nor was there an apparent reduction when commercial α-multistriatin was added at two different concentrations. We may therefore conclude that the use of a single

Table 1. Response of *Scolytus* species (mean number per trap and collecting period) to window traps baited with 4-methyl-3-heptanol (MH) and cubeboil (Cu) with or without addition of racemic α-multistriatin (a) or (-)-δ-multistriatin (d) at standard concentration (a, d) and at 10-fold dilution (a^{-1}, d^{-1}). Locations: forestry districts Whyl (W) and Rottweil (R).

	S. multistriatus		*S. scolytus*	
Bait	$\bar{x}$ (W)	$\bar{x}$ (R)	$\bar{x}$ (W)	$\bar{x}$ (R)
MH + Cu + a	11.7	18.3	10.5	12.5
MH + Cu + a^{-1}	2.9	5.9	17.7	13.3
MH + Cu + d	4.1	5.6	13.4	10.3
MH + Cu + d^{-1}	6.8	3.2	28.1	8.0
MH + Cu	2.2	4.9	12.5	11.9
Control (empty)	1.1	0.7	5.4	2.2
(n=)	(1,124)	(1,499)	(3,413)	(2,270)

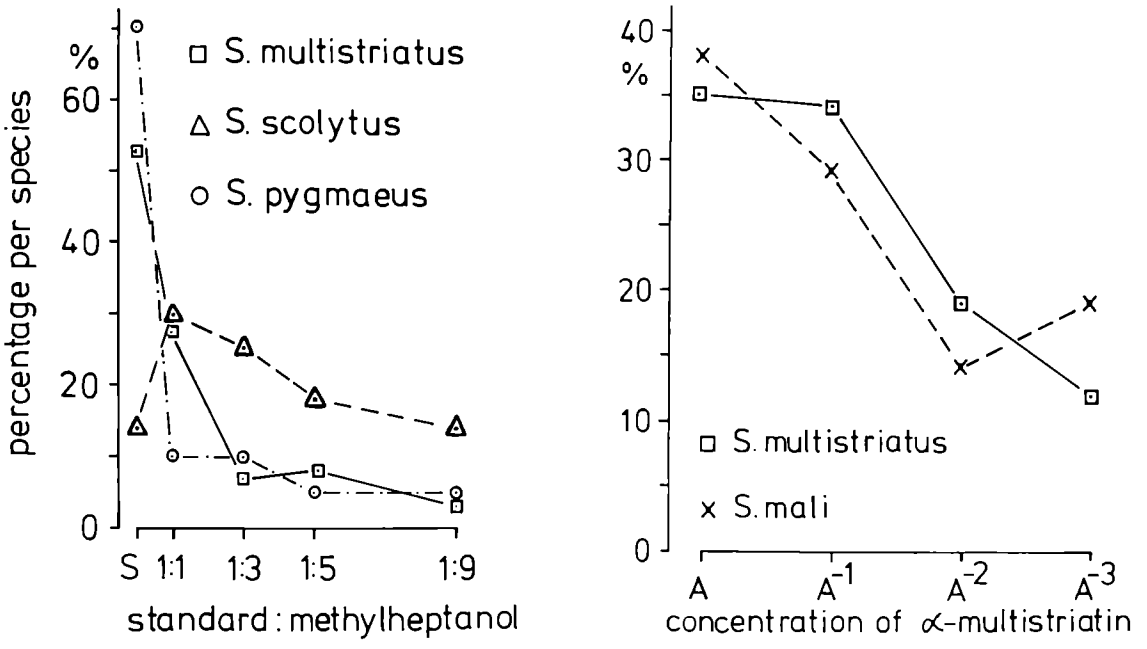

Figure 1. Response of *Scolytus* species to window traps baited with 4-methyl-3-heptanol, cubeboil and multistriatin (S) or racemic α-multistriatin (A) at standard concentration and subsequent dilutions of the whole bait (I) and of α-multistriatin only (II) (Schroth, 1978; Kopp, 1982).

bait containing α-multistriatin is feasible for both *Scolytus* spp. Inhibition induced by high concentrations or quantities of multistriatin seems to begin at a lower threshold for *S. scolytus* than for other *Scolytus* spp. Standard concentration of a commercial mixture of 4-methyl-3-heptanol, multistriatin and cubeboil ("multilure"), which was most attractive to *S. multistriatus* and *S. pygmaeus* (F.), inhibited response of *S. scolytus* to window traps (Figure 1). Dilution with 4-methyl-3-heptanol led to a marked increase of catches of *S. scolytus*; further dilutions, however, led to a gradual decrease in the number of beetles caught (Schroth, 1978). Also, *S. multistriatus* and *S. mali* Bechst. responded similarly to the dilution of α-multistriatin with 4-methyl-3-heptanol and cubeboil being released at a constant rate (Figure 1).

In *S. mali*, female beetles are responsible for host finding and the production of an aggregation pheromone (Rudinsky *et al.*, 1978). Both sexes of *S. mali* from natural or forced attacks contained varying amounts of 4-methyl-3-heptanol with *threo*- and *erythro*-diastereomers at a ratio of 1 : 5 for boring dust produced by males (Francke *et al.*, unpublished); female beetles contained a sex-specific compound so far unidentified (Figure 2). In field tests, billets artificially infested with *S. mali* females or uninfested billets of *Malus* sp. attracted *S. mali* and also *S. multistriatus*, whereas billets infested with male *S. mali* caught few beetles (Kopp, 1982). *S. mali* strongly responded to the attractant pheromone mixture of *S. multistriatus*, and reaction of both species to different α-multistriatin concentrations was similar (c.f. Figure 1). Reaction of *S. mali* to 4-methyl-3-heptanol alone or with addition of (-)-δ-multistriatin was distinctly lower. There is a considerable diurnal variation in flight activity between *S. mali* and *S. multistriatus* (Figure 3).

In some bark beetle species, attraction to the beetle-produced pheromone is inhibited by the ketone of an attractant alcohol, e.g. verbenone or methylcyclohexenone in *Ips typographus* (L.) or several *Dendroctonus* spp. We tested whether *Scolytus* species would be repelled by the ketone of 4-methyl-3-heptanol. In window traps, 4-methyl-3-heptanone did not inhibit the response of *S. scolytus* or *S. multistriatus* neither when offered alone or in combination with α-multistriatin, 4-methyl-3-heptanol and cubeboil (Table 2).

Evaluation of dispensers

Different types of dispensers for synthetic pheromones (Figure 4) were compared in window traps as to their performance. Glass capillaries containing single bait components were put in glass tubes with a polyethylene stopper to prevent rain water from

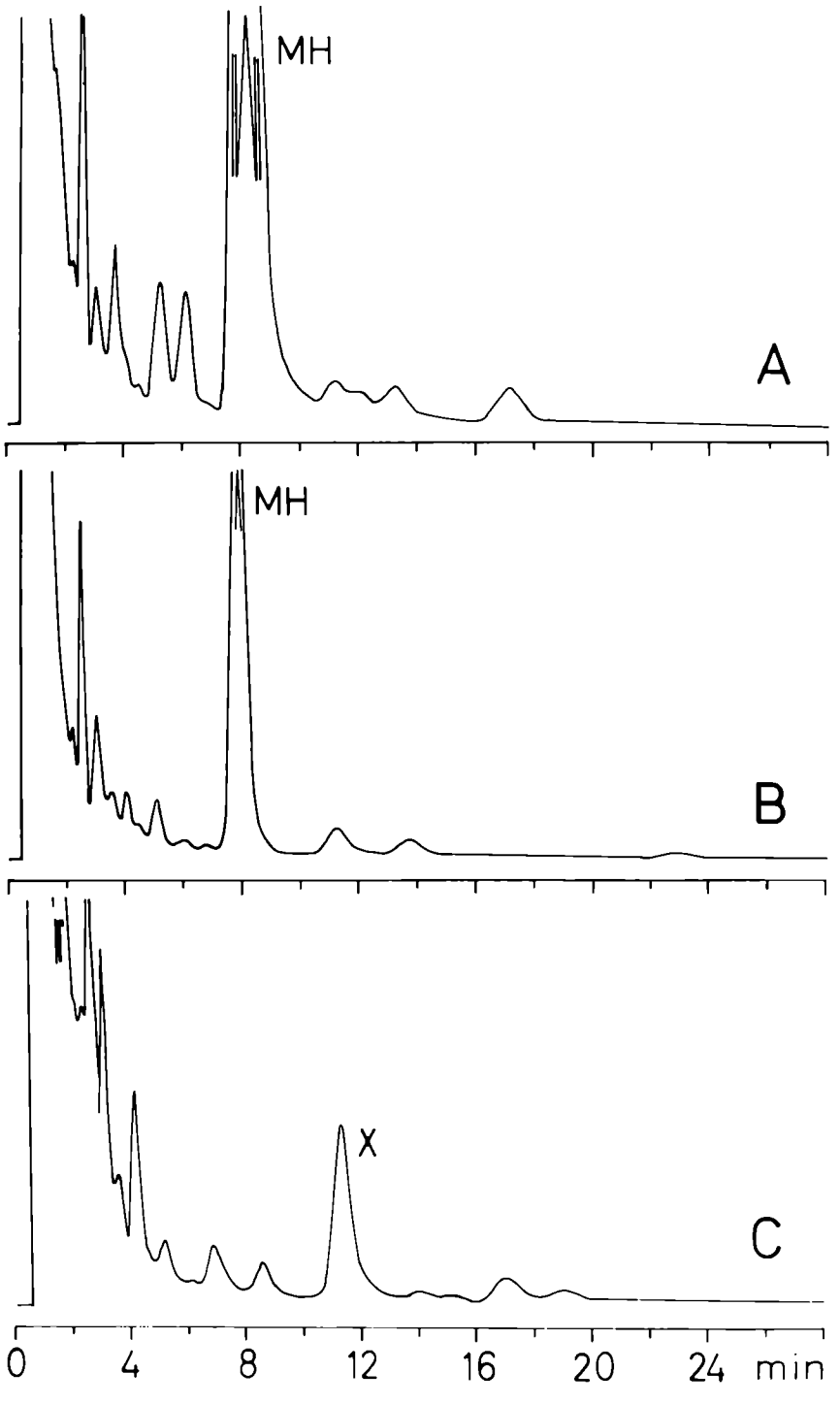

Figure 2. Gas chromatograms from frass of male (A) and female (B, C) *S. mali* artificially introduced into billets of *Malus* sp. (packed FFAP column, 100°C isotherm; MH = 4-methyl-3-heptanol, X = unidentified female specific compound).

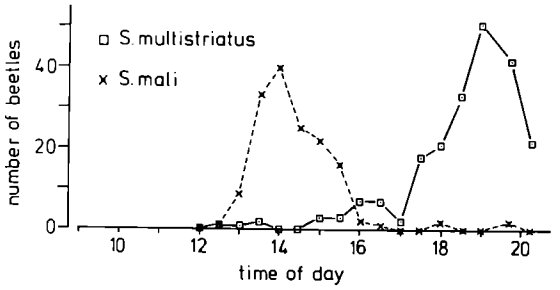

Figure 3. Flight pattern of *S. multistriatus* and *S. mali* at window traps baited with α-multistriatin, 4-methyl-3-heptanol and cubeboil.

entering; two holes in the stopper allowed the chemicals to evaporate. Mixtures of pheromone components in heptane were also tested in glass and plastic containers. Polyvinyl chloride (PVC) and glass tubes with perforated stopper proved comparable in the release of the standard mixture to capillaries containing the components separately. Release through polyethylene (PE) bags, however, caught distinctly less *Scolytus* species (Table 3).

Colonisation pattern of trap trees

We tested the effect of synthetic baits for attraction of *Scolytus* species to felled trap trees and also the

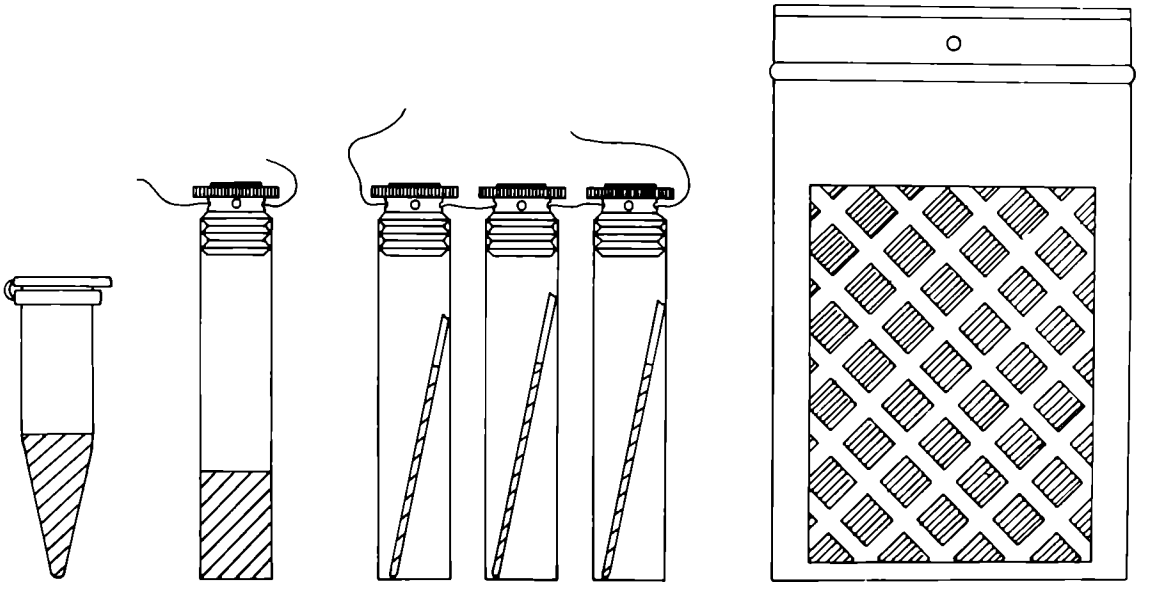

Figure 4. Types of dispensers used in the experiments: PVC-tubes, glass tubes with perforated stopper, standard capillaries and PE-bags.

Table 2. Response (%) of *Scolytus* species to window traps baited with the attractant pheromone mixture 4-methyl-3-heptanol (MH), cubeboil (Cu) and α-multistriatin (a) with or without addition of 4-methyl-3-heptanone.

Bait	Percentage of *Scolytus*		
	multistriatus	*scolytus*	*pygmaeus*
MH + Cu + a	22	28	22
MH + Cu + a	23	27	44
MH + Cu + a + 4-methyl-3-heptanone	21	14	11
MH + Cu + a + 4-methyl-3-heptanone	21	17	11
4-methyl-3-heptanone	7	9	6
Control (empty)	6	5	6
(n=)	(1,363)	(350)	(46)

Table 3. Response (%) of *Scolytus* species to window traps baited with 4-methyl-3-heptanol, cubeboil and α-multistriatin in various dispensers.

Dispenser	Percentage of *Scolytus*	
	multistriatus	*scolytus*
PVC-tube	28	32
Glass tube	27	36
Standard capillaries	26	22
PE-bag (0.9 ml)	11	3
PE-bag (1.8 ml)	8	7
(n=)	(281)	(59)

susceptibility of different elm species to beetle attack. Of the host trees *Ulmus carpinifolia* Gl., *U. glabra* Huds. and *U. laevis* Pall. which were put up alternately at distances of 15 – 20 m, only *U. carpinifolia* was (heavily) infested and the other two elm species hardly at all, regardless of whether baited with α-multistriatin, 4-methyl-3-heptanol and cubeboil or not (Figure 5). *S. scolytus* and *S. multistriatus*

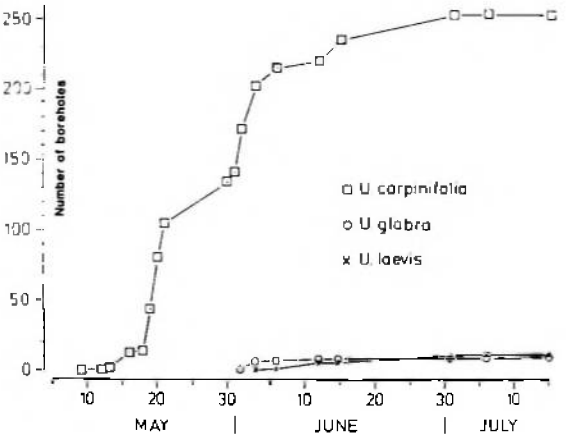

Figure 5. Number of bore holes of *Scolytus* species in felled trap trees of different elm species.

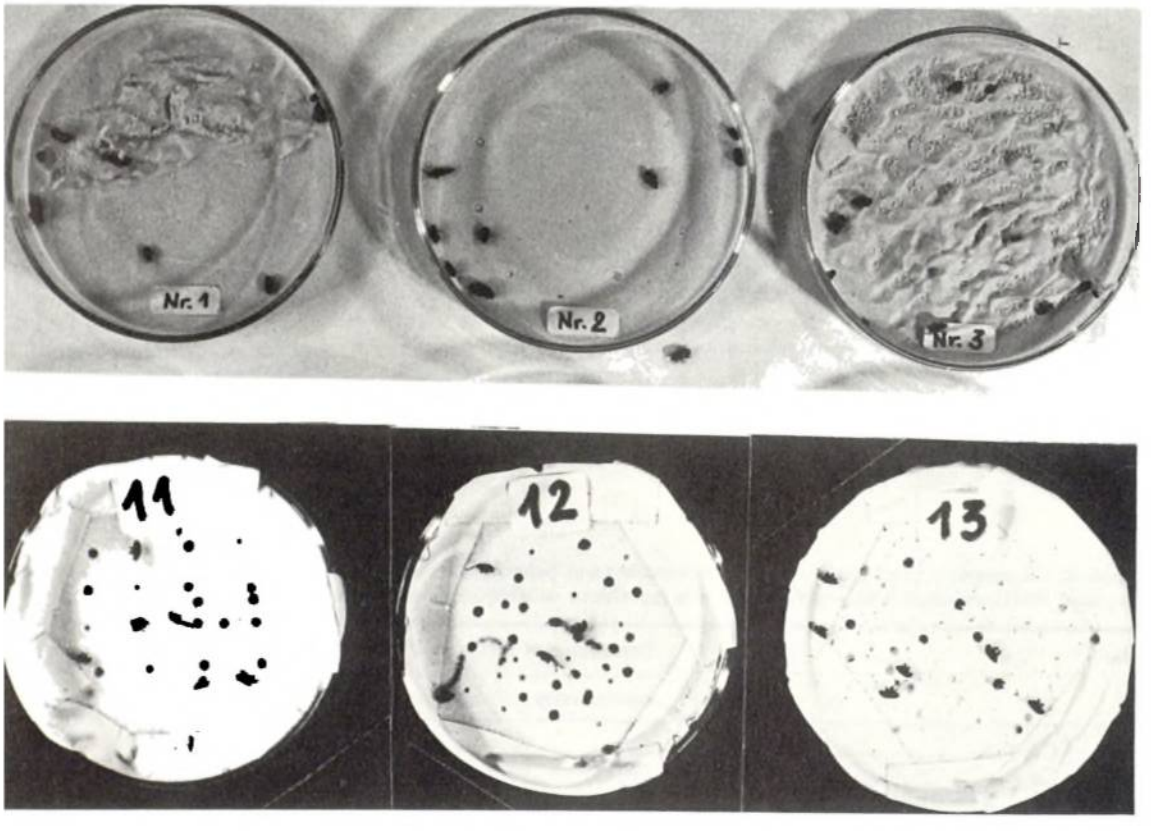

Figure 6. Feeding activity of *Scolytus* species in petri dishes, which were filled with plaster of Paris (1–3) or styropor (11–13). 1. no treatment, surface partly roughened; 2. drops of ethanol extract of *U. carpinifolia* added; 3. surface roughened, completely treated with extract of *U. carpinifolia*; styropor discs over pieces of bark from *U. glabra* (11), *U. laevis* (12) and *U. carpinifolia* (13).

artificially introduced into billets of these elm species differed accordingly with respect to their boring activity. However, ethanol extracts or pieces of bark did not elicit differential feeding behaviour when tested in the laboratory. Regardless of whether an ethanol extract of bark from *U. carpinifolia* was added or not, beetles started gnawing and boring into plaster of Paris only if the surface was roughened, and beetles readily bored into styropor discs put over pieces of bark regardless of elm species (Figure 6). These findings demonstrate the importance of beetle behaviour and host quality besides pheromone chemistry (Ascher and Guerevitz, 1972; Vité *et al.*, 1976; Grimm, 1977; Barger, 1979; von Keyserlingk, 1980; Svihra and Koehler, 1981). Extracts of *U. carpinifolia* slightly increased beetle catch in window traps (Schroth, 1978), but the performance of landing traps for *S. scolytus* and *S. multistriatus* could not be improved by addition of elm extracts to the synthetic pheromone or by using natural baits.

Pheromone traps

Regardless of the type of bait used, window traps were far more efficient than Norwegian cylinder traps. This relationship did not change during the course of the generation or from first to second generation, a phenomenon known from other scolytid species. In the landing traps, more of the host selecting sex of *S. scolytus* were caught, whereas the opposite is true for the flight barrier trap (Table 4).

Table 4. Total number of *Scolytus* species caught in two different types of traps baited with 4-methyl-3-heptanol, cubeboil and α- and δ-multistriatin. Locations: forestry districts Whyl (W) and Rottweil (R).

Species of beetle	Number of beetles caught in	
	perforated cylinders Σ (♂:♀)	window traps Σ (♂ : ♀)
S. multistriatus (W)	0	1,124 (1 : 1.5)
„ (R)	1 (1♀)	1,499 (1 : 1.6)
S. scolytus (W)	45 (1 : 0.6)	3,413 (1 : 1.4)
„ (R)	24 (1 : 0.8)	2,270 (1 : 1.4)

The number of beetles caught per trap was not normally distributed around the mean but showed a positive skewness (Figure 7); this is also the case for other types of traps and other bark beetle species and shows that trap efficiency could still be greatly improved.

For *S. scolytus* and *S. multistriatus*, the catch per window trap baited with the same amount of pheromone increased with the size of the flight

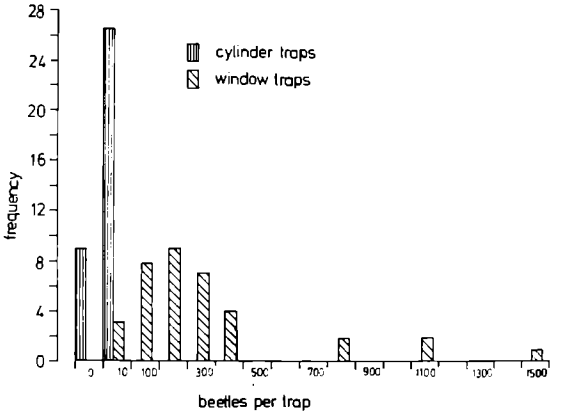

Figure 7. Catch frequency of *Scolytus* species per trap in Norwegian cylinder traps and window traps (Baader, 1981).

barrier (Figure 8). This relationship has also been demonstrated for sticky traps with *S. multistriatus* (Lanier *et al.*, 1976) and for the other bark beetle species and trap types.

Various types of traps were tested for their efficiency in catching *Scolytus* species. Window traps consist of a 55 x 60 cm polyethylene sheet; attracted beetles fly against the window and fall into a trough fixed underneath containing water and a detergent. Landing traps consist of (black) cylinders perforated with numerous holes; attracted beetles land on the rough surface, enter through these holes and fall into a ventilated collecting jar. Out of six types of traps (Figure 9), window traps equipped with a water trough caught the most beetles (90 per cent), perforated cylinders ("landing traps") the least (Table 5).

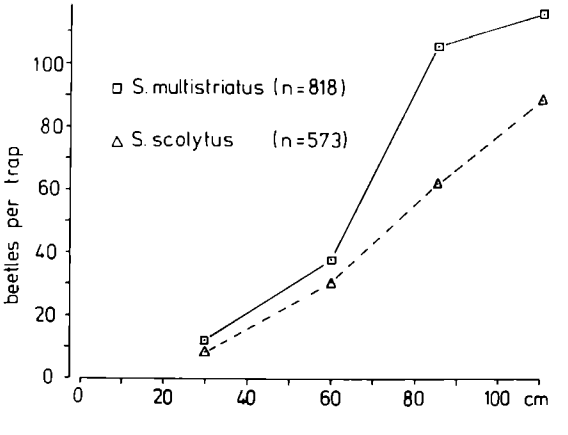

Figure 8. Amount of beetle catch per window trap using the same amount of pheromone, with increasing size of quadratic window (Schroth, 1978).

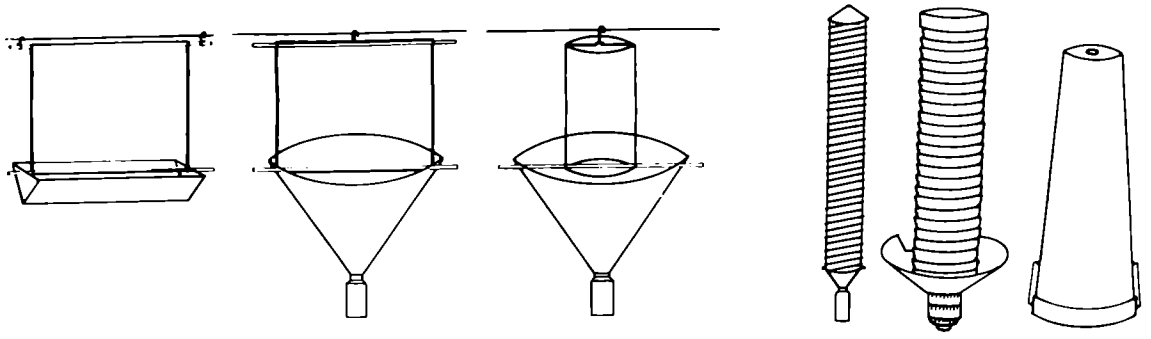

Figure 9. Types and designs of traps used in the experiments: window trap with water trough, funnel trap, funnel trap with cylindrical window, drainage pipe (diameter 10 cm), Norwegian cylinder trap (diameter 20 cm), and "Konus" trap.

Table 5. Response (%) of *Scolytus* species to various types of traps baited with 4-methyl-3-heptanol, cubeboil and α-multistriatin.

Type of trap	Percentage of *Scolytus*	
	multistriatus	*scolytus*
Window	92	89
Funnel	4	2
Funnel with cylindrical window	0	6
Drainage pipe	3	2
Norwegian cylinder (Borregaard)	0	2
"Konus" trap (Rochling-Haren)	0	0
(n=)	(306)	(66)

Table 6. Percentage of *Scolytus* species caught on sticky traps (mL) and traps not treated with sticky material (oL). Traps were baited with the attractant mixture released from capillaries.

Species/treatment		Window trap	Drainage pipe	Norwegian cylinder
S. multistriatus	oL	34	11	4
,,	mL	66	89	96
(n=)		(99)	(51)	(9)
S. scolytus	oL	15	0	0
,,	mL	85	100	100
(n=)		(87)	(10)	(1)

Table 7. Percentage of *S. multistriatus* caught in perforated cylinder traps painted black or white and, located inside or outside a forest stand.

Colour \ Site	Forest stand	Open field
Black	3	19
White	22	56

Flight barrier traps which attract and kill beetles are the most effective trap types. Experiments using landing traps covered with a sticky substance indicated that they can attract but not necessarily catch beetles (Table 6). Window traps, however, catch many other flying insects and debris, and need frequent cleaning. Various methods were therefore applied in the attempt to increase the performance of landing traps: tests with 10-fold higher concentrations of 4-methyl-3-heptanol and/or cubeboil, opening of additional holes (diameter 5 cm) to increase ventilation, or increasing the substrate humidity of landing traps made from plaster of Paris, and mimicking bark surface of elm trees (Figure 10) failed to increase the number of beetles entering the cylinders. Finally, an attempt was made to increase the optical component of tanding traps by locating them outside the forest and painting them white (Table 7). Results confirm lhat good visibility of traps strongly influences the beetle catch.

Figure 10. Norwegian cylinder trap with additional ventilation holes (diameter 5 cm) and painted white; landing trap with bark-like surface made from plaster of Paris and moisturized by means of a water container; and drainage pipe covered with sticky wire mesh.

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PAPER 11

Biochemical investigations related to Dutch elm disease carried out at the Agricultural Research Council Unit of Invertebrate Chemistry and Physiology, University of Sussex, 1973–1982.

J. F. GROVE

School of Molecular Sciences, University of Sussex, Falmer, Brighton, BN1 9QT, U.K.

INTRODUCTION

Problems involving biochemical interactions between an insect, a micro-organism and the plant host possess a particular fascination which stems from the integrated multidisciplinary approach required. The research potential of Dutch elm disease (DED), in which the vectors for the fungal pathogen *Ceratocystis ulmi* (Buisman) C. Moreau [= *Ophiostoma ulmi* (Buisman) Nannf.] are (in the U.K.) the bark beetles *Scolytus scolytus* (F.) and *S. multistriatus* (Marsham), was assessed in 1973. Many facets of this problem appeared likely to have a chemical basis and we elected to study:

I. THE SECONDARY METABOLITES PRODUCED BY *CERATOCYSTIS ULMI* ON SYNTHETIC AND ON ELM BARK/PHLOEM MEDIA.

In so far as it concerns phytotoxin formation by the aggressive and non-aggressive strains of *C. ulmi*, this aspect of our work is not reviewed here, but the relevant publications are listed (Claydon *et al.*, 1974a, 1974b, 1980; Grove and Pople, 1979). *C. ulmi* did not produce any of the known components of the aggregation pheromones (see section II A 1) of the beetle vectors (UICP, unpublished). However, the sterilizing process necessary for *in vitro* work removes from the natural media volatiles which could, conceivably, be precursors on the biosynthetic pathway to these components. A possible direct role for *C. ulmi* in beetle aggregation cannot therefore be completely excluded.

II. THE CHEMICALLY MEDIATED BEHAVIOUR OF THE BARK BEETLES

This work was begun in 1976 and three aspects (projects A–C) of beetle-host tree interaction are being investigated:

(A) The effect of volatiles released during gallery construction (boring).

(B) The effect of volatiles released during twig crotch feeding.

(C) The identification of host compounds, both volatile and involatile, concerned with the primary attraction of the beetle to the host.

Work on projects B and C is not yet complete and is not reported on here.

Project A has been studied at three response levels:

1. Long range responses in field trials for aggregation pheromone components. These trials were carried out in collaboration with the Forestry Commission, Entomology Branch, Alice Holt Lodge (see King and Fielding, this volume).

2. Short range responses in laboratory walking-arrestant bioassays.

3. Sensory responses of (i) individual antennal receptors (single olfactory cells) associated with the sensilla and (ii) whole antennae, by electroantennography (EAG).

In connection with project C:

4. Feeding responses in a laboratory phagostimulant bioassay are also being investigated.

Both beetle vectors have been studied though most work has been done with *S. scolytus*. When release of a particular compound was sex-specific, the response of both sexes was studied. Initially *Ulmus procera* (English elm) was used, but the programme has now been extended to other elm species of importance in the U.K. These are *U. glabra* (wych elm) and two varieties of *U. carpinifolia* (Wheatley, Cornish elm).

Highly purified compounds were used in all bioassays, including field trials, and the results of all biological experiments were subjected to rigorous statistical analysis. Where possible, a randomised block design, with re-randomisation within the blocks, was used in field trials. In this work individual components of attractant baits were released from separate vials. The normal method of release was by diffusion through polythene vials, but open glass vials and glass vial-within-a-vial dispensers

were sometimes used. Release rates were determined under standard conditions in the laboratory.

II A Volatiles released during gallery construction

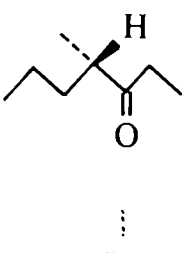
1. *The aggregation pheromones of the Scolytus sp. vectors.*

The potential of behaviour-controlling chemicals, particularly components of the aggregation pheromones, for the detection, population sampling and suppression of scolytid beetles is well established (Wood, 1979). In our work on the identification of the *Scolytus* sp. aggregation pheromones, the volatiles produced when unmated and mated male and female beetles bore into elm logs, under laboratory conditions, were trapped by conventional air entrainment techniques and identified by coupled gas chromatography—mass spectrometry (GCMS). The volatiles emitted by scored elm logs were similarly identified. Where necessary, larger quantities of these compounds were either synthesised or obtained from natural sources and were purified by chromatography. They were examined, either singly or in groups, by electro-physiological techniques (Section II A 3) and in the walking-arrestant bioassays (Section II A 2). Compounds found to be biologically active were then tested in the field.

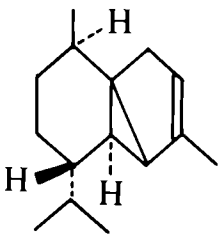
(i) BEETLE-PRODUCED VOLATILES

(a) *Scolytus multistriatus.* Soon after our work had been started, (-)-α-multristriatin (I) was identified (Pearce *et al.*, 1975; Lanier *et al.*, 1976) as one of the components of the unmated female-produced aggregation pheromone of North American populations of *S. multistriatus*. The other components were found to be (3S, 4S)-4-methylheptan-3-ol (V)† and a host synergist, the *U. americana* sesquiterpene (-)-α-cubebene (X). A small amount of the β-isomer (II) of multistriatin was also identified among the volatiles produced by female beetles. Of the four synthetic racemic stereoisomers, α(I), β(II), γ(III)

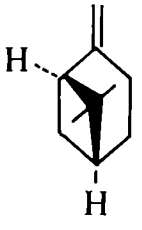
†=(-)-threo-4-methylheptan-3-ol



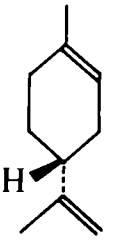
α-
I



β-
II

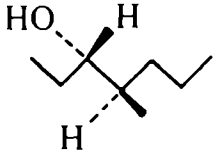


γ-
III

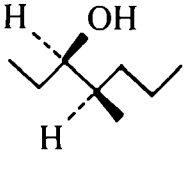


δ-
IV

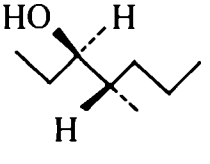
MULTISTRIATIN ISOMERS



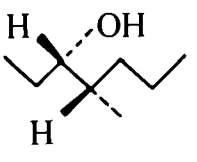
(3S, 4S)
V



(3R, 4S)
VI

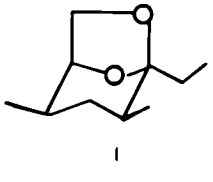


(3R, 4R)
VII

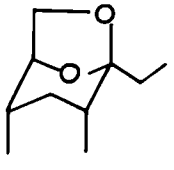


(3S, 4R)
VIII

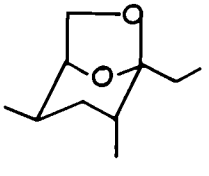
4-METHYLHEPTAN-3-OL ISOMERS



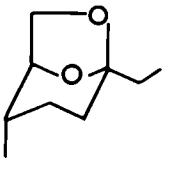
IX
(4S)-4-METHYL-
HEPTAN-3-ONE



X
(-)-α-CUBEBENE



XI
(-)-β-PINENE



XII
(-)-LIMONENE

and δ(IV), of multistriatin, only the α-isomer (I), in admixture with racemic 4-methylheptan-3-ol and cubebene, was found to attract *S. multistriatus* in both laboratory and field tests; the other isomers were inactive (Lanier *et al.*, 1977). A bait (multilure) containing all four multistriatin stereoisomers together with racemic 4-methylheptan-3-ol and distilled cubeb oil (70% α-cubebene) was effective in trapping N. American *S. multistriatus* populations (Lanier *et al.*, 1976). European (Upper Rhine valley) populations of *S. multistriatus* were also attracted to this bait (Vité *et al.*, 1976). It was claimed, initially (Gerken *et al.*, 1978), that these populations were attracted to (-)-δ-multistriatin (IV) admixed with methylheptanol and cubebene: the addition of (-)-α-multistriatin was said to reduce the attraction and when (-)-δ-multistriatin was replaced by the (-)-α-isomer, the mixture was inactive. However, in further work with these same beetle populations it was subsequently found (Klimetzek *et al.*, 1981) that mixtures of α-multistriatin, methylheptanol and cubeb oil were attractive, but (-)-δ-multistriatin was inactive. Blight *et al.*, (1980b) had previously shown that English populations of *S. multistriatus* were attracted to baits containing (-)-α-multistriatin, the (+)-α-isomer being inactive, and had identified (UICP, unpublished) α-multistriatin from English female *S. multistriatus* boring into *U. procera*. Field trials in England with methylheptanol baits to which were added (+)- or (-)-β- or racemic α-, γ- or δ-multistriatin (Blight *et al.*, 1983a) confirmed the results of the American workers and there is now little doubt that the components of the aggregation pheromones of American and European populations of *S. multistriatus* are identical.

Although low release rates of α-multistriatin greatly enhanced the response of *S. multistriatus* to baits containing methylheptanol and a host synergist, the release of larger amounts interrupted (*sensu* Wood, 1977) the response (Blight *et al.*, 1983a). This work suggested that the multilure bait is probably not the optimum formulation for attracting *S. multistriatus*. Male *S. multistriatus* did not produce measurable amounts of either α-multistriatin or methylheptanol (UICP, unpublished), and did not appear to contribute to the aggregation of this species (Peacock *et al.*, 1971).

4-Methylheptan-3-one (IX), formally identified from male *S. scolytus* (see below), was also produced by female *S. multistriatus* (Blight *et al.*, 1983b). In a field trial, a low release rate of the racemic ketone did not significantly increase the catch of a methylheptanol bait, whilst a high release rate reduced the catch (Blight *et al.*, 1982).

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The most important conclusion to be drawn from the field experiments reviewed above is that one bait, based on methylheptanol and incorporating a low release rate of α-multistriatin, can be formulated to capture both beetle vectors.

† = (–)-erythro-4-methylheptan-3-ol

Forestry Commission

Bulletin 60

Research on Dutch elm disease in Europe

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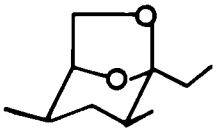
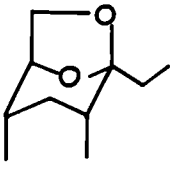
CORRECTION

Page 60

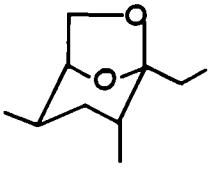
Artwork for the chemical configurations was regrettably transposed. The correct diagrammatic representation appears below:



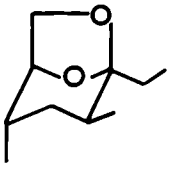
α–
I



β–
II

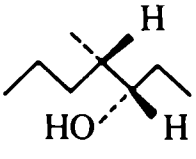


γ–
III

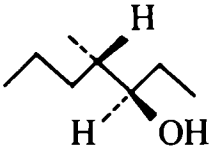


δ–
IV

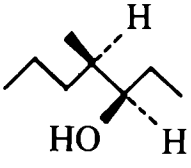
MULTISTRIATIN ISOMERS



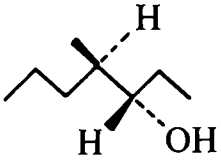
(3S, 4S)
V



(3R, 4S)
VI

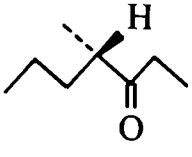


(3R, 4R)
VII

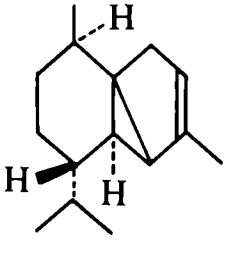


(3S, 4R)
VIII

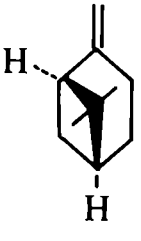
4-METHYLHEPTAN-3-OL ISOMERS



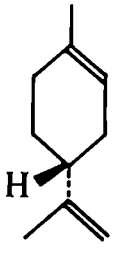
IX
(4S)-4-METHYL-
HEPTAN-3-ONE



X
(–)-α-CUBEBENE



XI
(–)-β-PINENE



XII
(–)-LIMONENE

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London: Her Majesty's Stationery Office

and δ(IV), of multistriatin, only the α-isomer (I), in admixture with racemic 4-methylheptan-3-ol and cubebene, was found to attract *S. multistriatus* in both laboratory and field tests; the other isomers were inactive (Lanier *et al.*, 1977). A bait (multilure) containing all four multistriatin stereoisomers together with racemic 4-methylheptan-3-ol and distilled cubeb oil (70% α-cubebene) was effective in trapping N. American *S. multistriatus* populations (Lanier *et al.*, 1976). European (Upper Rhine valley) populations of *S. multistriatus* were also attracted to this bait (Vité *et al.*, 1976). It was claimed, initially (Gerken *et al.*, 1978), that these populations were attracted to (-)-δ-multistriatin (IV) admixed with methylheptanol and cubebene: the addition of (-)-α-multistriatin was said to reduce the attraction and when (-)-δ-multistriatin was replaced by the (-)-α-isomer, the mixture was inactive. However, in further work with these same beetle populations it was subsequently found (Klimetzek *et al.*, 1981) that mixtures of α-multistriatin, methylheptanol and cubeb oil were attractive, but (-)-δ-multistriatin was inactive. Blight *et al.*, (1980b) had previously shown that English populations of *S. multistriatus* were attracted to baits containing (-)-α-multistriatin, the (+)-α-isomer being inactive, and had identified (UICP, unpublished) α-multistriatin from English female *S. multistriatus* boring into *U. procera*. Field trials in England with methylheptanol baits to which were added (+)- or (-)-β- or racemic α-, γ- or δ-multistriatin (Blight *et al.*, 1983a) confirmed the results of the American workers and there is now little doubt that the components of the aggregation pheromones of American and European populations of *S. multistriatus* are identical.

Although low release rates of α-multistriatin greatly enhanced the response of *S. multistriatus* to baits containing methylheptanol and a host synergist, the release of larger amounts interrupted (*sensu* Wood, 1977) the response (Blight *et al.*, 1983a). This work suggested that the multilure bait is probably not the optimum formulation for attracting *S. multistriatus*. Male *S. multistriatus* did not produce measurable amounts of either α-multistriatin or methylheptanol (UICP, unpublished), and did not appear to contribute to the aggregation of this species (Peacock *et al.*, 1971).

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The most important conclusion to be drawn from the field experiments reviewed above is that one bait, based on methylheptanol and incorporating a low release rate of α-multistriatin, can be formulated to capture both beetle vectors.

† = (-)-erythro-4-methylheptan-3-ol

Ethanol, a volatile produced by infestations of *S. scolytus*, did not contribute to the aggregation pheromone when tested in the field (Blight *et al.*, 1980a). Likewise, the addition of racemic 4-methylheptan-3-one to a methylheptanol bait did not significantly increase the catch (Blight *et al.*, 1982), and high release rates of the ketone reduced it. (+)-(4S)-4-Methylheptan-3-one (IX) had previously (Blight *et al.*, 1983b) been formally identified as a product when male, but not female, *S. scolytus* bore into the four *Ulmus* spp. studied.

(c) *Interactions between the beetle vectors.* From present knowledge it appears that α-multistriatin has multiple functions in the field behaviour of the two *Scolytus* spp. In addition to functioning as a component of the aggregation pheromone of *S. multistriatus*, α-multistriatin may play a role in interspecific attraction between the species. *S. multistriatus* was attracted to billets artificially infested with both sexes of *S. scolytus* (Blight *et al.*, 1980a). Under these conditions 4-methylheptan-3-ol and α-multistriatin are simultaneously produced by male and female beetles respectively (UICP, unpublished). Thus, *S. scolytus*, which in southern England normally emerges earlier in the season, produces, when colonising stressed elm, a pheromone bouquet similar in composition to multilure, which attracts the sympatric *S. multistriatus*.

The release of large amounts of α-multistriatin may be part of a spacing mechanism for both species, by which beetle attack is shifted to less heavily colonised areas of the same tree or to other trees. The known production of α-multistriatin, but little or no methylheptanol, by mated female *S. multistriatus* (Gore *et al.*, 1977) supports this hypothesis.

(d) *Biosynthesis of the beetle-produced volatiles.* As part of a wider investigation into the source of the beetle-produced components of the aggregation pheromones, we examined the gut microbial flora of *S. scolytus* at all stages of its development (Burges *et al.*, 1979). Because the formation of these components is likely to involve oxidative processes, strict obligate anaerobes were not sought. Adult *S. scolytus* emerged from the pupal stage with sterile guts (although micro-organisms were present within the pupal body) and in general, gut sterility was maintained whilst boring out of an elm billet. The adult gut microbial flora was mainly acquired during subsequent feeding. Differences were found between the gut flora of both larval and adult *S. scolytus* and of male and female beetles. The most ubiquitous organisms, found in both sexes, were *Corynebacterium* sp. and the yeast *Candida rhagii*. Other yeasts and Actinomycetes were also present and *Serratia rubidaea* appeared to be male-specific (Burges *et al.*, 1979). Neither multistriatin nor methylheptanol were produced when these organisms were grown on natural media derived from elm bark or phloem (UICP, unpublished).

(e) *The effects of juvenile hormone.* The amount of α-multistriatin produced by fed female beetles was unaltered by topical application of JH III, but in fed males the amount of 4-methylheptan-3-ol was greatly increased and the production of α-multistriatin was induced (Blight *et al.*, 1979a).

(ii) HOST-PRODUCED VOLATILES

The changes induced by gallery construction in the volatiles emanating from an elm billet were quantitative rather than qualitative, but the pattern of volatiles detected was characteristic of the elm species (UICP, unpublished).

A number of *U. procera* volatiles elicited antennal responses (Section II A 3) in *S. scolytus* beetles, including the monoterpenes (-)-β-pinene (XI) and (-)-limonene (XII) and the sesquiterpene (-)-α-cubebene (X) (Wadhams, 1982; UICP, unpublished). In an early field trial (Blight *et al.*, 1978a) with methylheptanol baits the addition of α-cubebene significantly increased the catch of *S. scolytus*, but more recent trials (Blight *et al.*, 1979c, 1980a) have not confirmed this result. It is now clear (UICP, unpublished) that release rate, and perhaps release ratio, is an important factor in the activity of this host synergist. With a low release rate, more beetles were caught than with methylheptanol alone, but there was a significant decrease in the number caught by the combined bait when the release rate was increased. Similar results have been obtained with *S. multistriatus*.

α-Cubebene is scarce and expensive and a number of trials have been carried out with (-)-β-pinene and (-)-limonene as potential substitutes in baits used for mass trapping/population sampling. The antennal club of *S. scolytus* has cells which respond specifically to β-pinene, and probably also to limonene; and walking beetles respond to these compounds when each is presented in combination with methylheptanol (see below). However, field trials with these compounds have been inconclusive (Blight *et al.*, 1980a; UICP, unpublished). In the most recent trial, in 1981 (UICP, unpublished), α-cubebene, limonene and β-pinene were tested (in conjunction with methylheptanol) singly, in pairs, and as the triple combination, using release rates which, from the results of earlier trials, were considered to be optimum when the compounds were released individually with methylheptanol. When the results were analysed none of the host volatiles had a significant effect on the catch of either beetle vector. The reason for these conflicting results is unclear.

As the trials were conducted in the vicinity of diseased elms, the air may already have been 'saturated' with host volatiles, and the additional release of these compounds may then have had a minimal effect.

2. *Short-range responses*

Initially (Blight *et al.*, 1978b) a walking-beetle bioassay (Peacock *et al.*, 1973) was used in which the test compound, applied to a filter paper disc below a runway, induced arrestant and turning responses. This bioassay was employed successfully to monitor *S. scolytus* air entrainment extracts and to demonstrate the response of *S. scolytus* beetles of both sexes to 4-methylheptan-3-ol (Blight *et al.*, 1978b). Subsequently, a more sophisticated laminar air flow bioassay, modified from that developed by Moeck (1970), was used to study beetle response to 4-methylheptan-3-one (Blight *et al.*, 1983b) and to α-multistriatin and its enantiomers (UICP, unpublished). A beam of light, at right angles to the air stream, induced beetles to cross a 2 cm wide plume of the compound being tested. The responses were recorded graphically and classified according to type, such as upwind or downwind deflection, arrival at plume source, etc.

4-Methylheptan-3-one, alone, was only weakly active, but in combination with methylheptanol and α-cubebene, the mixture was significantly more attractive to female *S. scolytus* beetles than the methylheptanol-cubebene standard. The dose-response curve passed through a maximum. The response of male beetles to methylheptanol-cubebene was not significantly altered by the addition of methylheptanone. 4-Methylheptan-3-one thus appears to have a sex identifying role and attracts walking female beetles at short range. High concentrations of α-multistriatin were likewise attractive to male *S. scolytus* beetles in the presence of methylheptanol and cubebene. Although neither the (+) nor (-)-enantiomers produced a significantly greater *overall* level of response than the methylheptanol-cubebene standard in males or females, differences in the intensity and type of response suggested that *S. scolytus* could detect both enantiomers (see below).

Preliminary experiments (UICP, unpublished) have suggested that both α-cubebene and β-pinene enhance the short range response of *S. scolytus* to methylheptanol.

3. *Sensory responses*

The morphology of the antennal clubs of both male and female *S. multistriatus* and *S. scolytus* has been studied by scanning electron microscopy and the abundance and distribution of the sensilla have been determined (Henderson and Wadhams, 1981). The antennal clubs resembled those of other, previously studied (Payne *et al.*, 1973) Scolytids, and two types of sensillum basiconicum, two types of sensillum trichodeum, sensillum chaetica (mechanoreceptors) and fluted sensilla were found in both species. The sensilla were evenly distributed on both faces of the club except for trichodea type III which were more common on the anterior face in both species. Two crescent-shaped sensillum-filled depressions (fields 1 and 2), separated by an area devoid of sensilla encircled the basal half of the club, whilst the distal half was less densely covered and constituted a third field. Sensillum bascionicum long type was the most numerous type of sensillum in both species. In *S. multistriatus* long basiconica were predominant in all three fields, whilst in *S. scolytus*, trichodea type II were more abundant in field 3. No major intersexual differences were apparent in either species (Henderson and Wadhams, 1981). In preliminary experiments there was no obvious correlation of sensillum type with response to specific components of the aggregation pheromones (UICP, unpublished).

Antennae of both male and female *S. scolytus* responded to all four stereoisomers of 4-methylheptan-3-ol, but stronger responses were given by both sexes to the (-)-threo- and (-)-erythro-alcohols (Blight *et al.*, 1979b), reflecting the known formation and field activity of these compounds. EAG and single cell recording techniques demonstrated the presence of separate receptors for (-)-threo- and (-)-erythro-4-methylheptan-3-ol on the antennae (Wadhams *et al.*, 1982). The majority of these "single cell" recordings showed spikes of two different amplitudes, arising from two different cells. The cell giving spikes of larger amplitude responded to the (-)-threo isomer whilst the cell with spikes of smaller amplitude responded to the (-)-erythro isomer. The two cells appeared to be associated with a single long sensillum basiconicum. These cells did not respond to any of the other beetle or host volatiles, except at very high stimulus concentrations.

Antennae of both male and female *S. scolytus* responded to *both* enantiomers of α-multistriatin but dose-response curves showed that there was a clear antennal discrimination in favour of the (-)-enantiomer (UICP, unpublished). As with the (-)-isomers of methylheptanol, (-)-α-multistriatin was perceived by a separate group of specialised olfactory cells, but there was also another group of cells which responded to α-multistriatin but only at relatively high stimulus concentrations. These cells responded preferentially to the (+)-enantiomer and were presumably responsible for the EAG dose-response curve obtained with this compound.

The *S. scolytus* EAG threshold for 4-methylheptan-3-one was higher than that for the (-)-isomers of methylheptanol and there was no significant difference in the response by male and female beetles. As with the alcohols, low concentrations of the ketone activated a specific group of cells. Higher stimulus concentrations of other pheromone components also elicited a response from these cells but only at concentrations at which the methylheptanone dose-response curve had already saturated (Blight *et al.*, 1983b).

The EAG dose-response curves for the host volatiles (-)-α-cubebene, (-)-β-pinene and (-)-limonene were very similar and the threshold concentrations were high compared with that for 4-methylheptan-3-ol (UICP, unpublished). Although this EAG similarity could have arisen from all three compounds interacting with the same receptor site, the single cell technique revealed that separate groups of cells existed which responded specifically to α-cubebene, β-pinene and limonene respectively at low concentrations, and to none of the other compounds tested. However, these cells constituted a relatively small proportion of the total recorded, reflecting, no doubt, the relative importance of host volatiles in overall behaviour.

The majority of the cells recorded so far have been specific to the (-)-isomers of 4-methylheptan-3-ol, to 4-methylheptan-3-one, or to α-multistriatin, but there is a total of *ca.* 10 per cent which do not respond either to these beetle-produced compounds or to the above host volatiles (Wadhams, 1982). These cells are stimulated by the total air entrainment extract and are expected to respond to constituents of this extract which have hitherto remained unidentified because their EAG response thresholds are too high (UICP, unpublished). To assist the solution of this problem a coupled GC-single cell technique has been developed (Wadhams, 1982). This technique is more sensitive than coupled GC-EAG, and interesting and valuable results are expected.

It appears that possibly 11 or 12 physiologically-active compounds may be involved in the chemically-mediated behaviour of *S. scolytus*. This is undoubtedly a more complex situation than was believed likely when this investigation was started. The sensory responses of *S. multistriatus* have still to be investigated.

ACKNOWLEDGEMENT

The author is indebted to his colleagues Margaret M. Blight and Dr. L. J. Wadhams for helpful comments on the manuscript.

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PAPER 12

Trap design and experimental layout in pheromone research in Britain

C. J. KING and N. J. FIELDING
Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH, U.K.

Introduction

In 1977 a programme of research was initiated to investigate the aggregation pheromone of the larger European elm bark beetle *Scolytus scolytus* (F.), one of two vectors of Dutch elm disease in the United Kingdom. This research involved chemists of the Agricultural Research Council Unit of Invertebrate Chemistry and Physiology — ARCUICP — and entomologists of the Forestry Commission's Research and Development Division. The former body was responsible for all aspects of the insect's aggregation chemistry and laboratory studies of potential attractants, the latter body carried out all the field experimentation generated by the ARCUICP and also collected biological data on the aggregation behaviour of *S. scolytus*. Additional information was also collected by both groups on aspects of the aggregation chemistry and behaviour of the smaller European elm bark beetle *Scolytus multistriatus* (Marsham).

This paper describes briefly the experimental designs and equipment employed during 5 years of field experiments. Comments are also made on trap siting and climatic factors affecting this work.

Field experiments

Siting

The major pre-requisite for experimental sites is the presence of large numbers of breeding elm bark beetles present in infested elms evenly distributed throughout the area. In 1977, when this project began, the majority of elms in central southern England were already decimated. Therefore experiments were sited in discrete elm populations on the periphery of the denser original population. Without exception these sites were situated upon privately owned agricultural land and this necessarily confined the vast majority of field experiments to field edges amongst arable crops. This restriction made the layout of traps in homogeneous situations most difficult and exacerbated the trap/site effects which were encountered throughout; these are discussed later.

Climate

The climatic requirements for flight and distribution of *S. scolytus* are of infrequent occurrence in the average summer experienced in southern Britain. The success of the field work depended entirely on beetle flight and subsequent capture on baited traps. Initially experimental design took little account of this factor but as experience increased so designs were adapted to lessen the effects of irregular beetle flights and variable insect populations; these are discussed later.

Trap types

Throughout the experiments, with only one exception, sticky traps were employed. These were always designed to give a vertical silhouette crudely simulating a tree stem in outline. Due to the large numbers of traps employed, simplicity of design, cost and portability were all important considerations. At first a commercial sticky medium was used but from 1978 onwards a 'home made' sticky gel was used. This was of 'Hyvis 10' (B.P. Chemicals), a low density polybutenol bulked with polyethylene in a 94 per cent : 6 per cent volume/volume ratio, (Blight *et al.*, 1980). Captured elm beetles were hand picked from the traps and cleaned in high flashpoint petroleum (a solvent for the sticky gel). They could then readily be identified and sexed.

Trap/site effects

These are commonly found in many pheromone experiments where traps in a particular situation either fail to capture the target insect or capture far larger numbers than expected, often on unbaited traps. They can seriously confuse interpretation of the data and must be minimised if at all possible.

In the case of the experiments on *S. scolytus*, biased trap positions could, after some experience, be attributed to certain factors. Proximity to emerging beetle broods, placement downwind of a nearby natural pheromone source (recently attacked brood elm) or positioning a trap in a sheltered

sunny place, all led to much higher beetle capture than anticipated. Traps placed in dense shade or in highly exposed positions seldom caught significant numbers of beetles even with recognised attractants.

Other results were not easily explained or attributable to any observable site factor. When laying out experiments homogeneity within blocks was always the main criterion. The need for even spacing between traps to minimise possible trap interaction, and the constraints of using hedgerows and field margins, frequently made ideal trap siting impossible.

Experimental techniques and trap modifications

1977

The first field experiment was designed to find the pheromone producing sex of *S. scolytus* and utilised live beetles in elm billets within a mesh cylinder sticky trap (see Plate 1). The main drawbacks were expense and bulk, difficulty in applying the sticky coating (by brush when heated) and in stripping the captured beetles from woven metal mesh. These traps are, however, robust and durable though difficult to clean for re-use.

The experimental design was a totally randomised unblocked array with re-randomisation on a time basis. The main drawbacks to this design were: first, the difficulty in moving whole traps containing elm logs and live beetles over farmland and second, the predetermined randomisation periods took no account of actual beetle flight activity.

The mesh cylinder traps were also used with artificial baits which were wired to the supporting post (see Plate 2). These experiments with artificial baits employed a randomised block design with re-randomisation to reduce trap/site effects experienced in the first experiments.

1978

By 1978 a number of chemical compounds evaluated by the ARCUICP required field testing. It was decided to employ blower olfactometer traps to regulate and improve release rates of potential beetle attractants. Sixty of these traps were built and employed in field trials (Blight *et al.*, 1979). These were constructed of standard PVC pipe and couplings with a small battery driven fan passing a steady (but unmeasured) airflow over the baits, exiting through evenly spaced holes at the top. The latter was surrounded with a sticky cylinder of aluminium mesh (see Plate 3 and Figure 1). There was no evidence that this trap was superior to static types and it was expensive and extremely bulky. It also required constant attention to activate the fan motors and replace batteries and after initial use it was only employed in a static mode. The

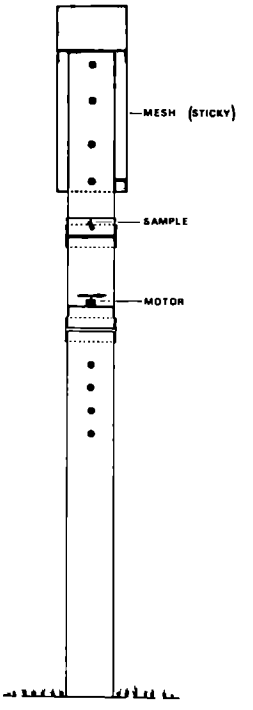

Figure 1 Blower olfactometer trap design

original mesh cylinders were also used with artificial baits during this year.

The experimental designs used were all randomised blocks with replication governed by the number of treatments and traps available, which normally allowed six blocks in each experiment. As replication was high there was no re-randomisation, especially as the redeployment of bulky traps made this most difficult. Despite the size of the experiments, trap/site interactions markedly affected the experimental data.

1979

It was now clear that trap/site effects were a major consideration in experimental layout and design and whilst the cause of some unexpected results could be explained and the causal situations largely avoided, others could not. The constant constraint of using field edges and hedgerows made optimum trap positioning impossible. It was hoped to ameliorate such effects by increasing experimental replication and this created a need for a lightweight, cheap and effective sticky trap. This resulted in the design and manufacture of a vertical fin trap made of two interlocking sheets of grey PVC slotted into cuts sawn at right angles into the top of wooden posts (see Plate 4). A central hole in the

fin allowed chemical baits to be hung and for the prevailing air current to pass over them. To increase their visual contrast the traps had white PVC strips added to their outer edges.

Experimental design was that of exclusively randomised blocks with ten replications, intended to even out trap/site effects. Whilst this was achieved in many cases some results were still clouded by such effects. The trap design proved easy to manage though the bulked traps were of considerable weight. Being flat surfaced they were easy to coat with the sticky medium and easy to strip of beetles and clean. They also stood up well to high winds.

1980

Again methods of increasing experimental sensitivity were sought. Results to date had shown that differences between treatments were marginally significant and that the presence of biased traps, even few in number, tended to obscure such differences. Furthermore the rapid demise of the elms on suitable sites made it necessary to design experiments to fit within a smaller area.

Randomised blocks were established, usually of five replications, with re-randomisation within blocks after significant beetle capture. This method made best use of space and exploited sporadic beetle flights to the utmost. To facilitate this method the grey PVC fin traps were used in conjunction with disposable lightweight white plastic sticky covers (see Plate 5). Baits and covers could be rapidly changed with each new re-randomisation and in this way over 20 replications were possible from only five blocks. This method significantly reduced trap/site effects though the re-randomisation occasionally left one treatment in the same position.

1981

Methods used in 1981 were largely the same as in 1980 but the fin trap was made of white PVC corrugated sheets stapled to posts (see Plate 6). These were very light in weight and cheap enough to be disposable. They could also be removed in bulk to the laboratory for stripping of beetles and assessment. Despite their flimsy nature they were seldom damaged by extremes of weather. The only innovation in experimental design was to re-randomise without replacing in the same position. This gave an optimum of 25 replicates, with five blocks of five treatments which were repositioned five times within their blocks.

Discussion

It is felt that the equipment and experimental design used in 1981 are adequate in our field conditions. Any further attempt to increase the sensitivity of experiments would appear to lie in increasing the number of observations made. Undoubtedly the efficacy of sticky traps may be questioned as they offer attracted beetles no further options in behaviour, and indiscriminately capture all invertebrates that come into contact with them.

In 1980 a type of perforated cylinder trap was tested against *S. scolytus*. This 'Icopal' trap (see Plate 7) mainly captured beetles stimulated to enter the holes in the cylinder and also those falling from the outside of the trap. The baits used did attract flying beetles to the traps, but few were arrested or stimulated to enter the trap; it was thought that this trap design was ahead of our understanding of the insect's chemically mediated behaviour.

Conclusions

In Britain conditions of climate and diminishing elm populations conspire to make pheromone experiments on elm bark beetles difficult. Irregular beetle flight in a rapidly changing climate, limited field staff, siting restrictions and increasingly unsuitable elm populations will make large formal experiments impractical in the near future. To effect improvements in techniques we require both a greater knowledge of the target insect's biology, and the benefit of results from further experiments in trap design, height and placement.

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Plate 1 Mesh cylinder sticky trap containing an elm billet with live *Scolytus scolytus* bark beetles as bait

Plate 2 Mesh cylinder sticky trap with artificial bait

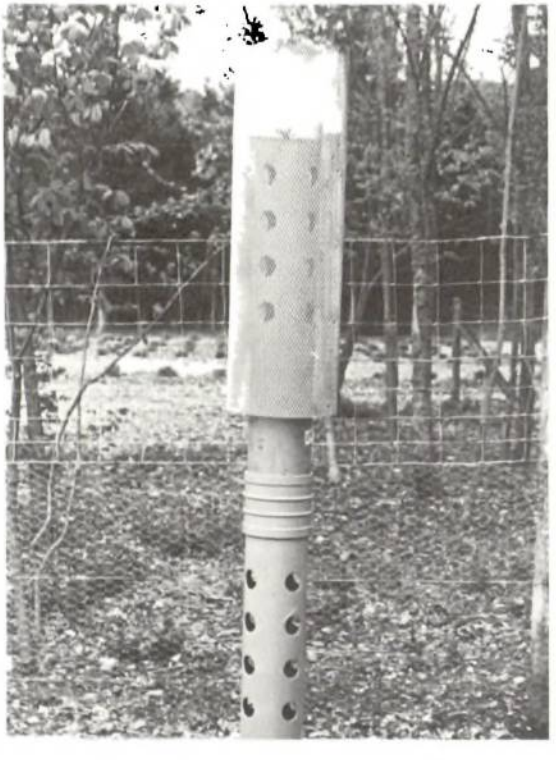

Plate 3 Blower olfactometer trap

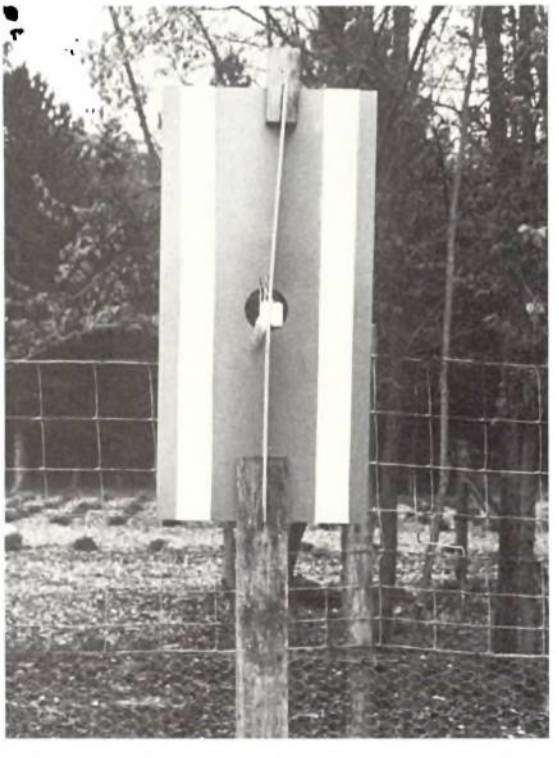

Plate 4 Vertical fin trap of grey PVC with outer white band on each fin

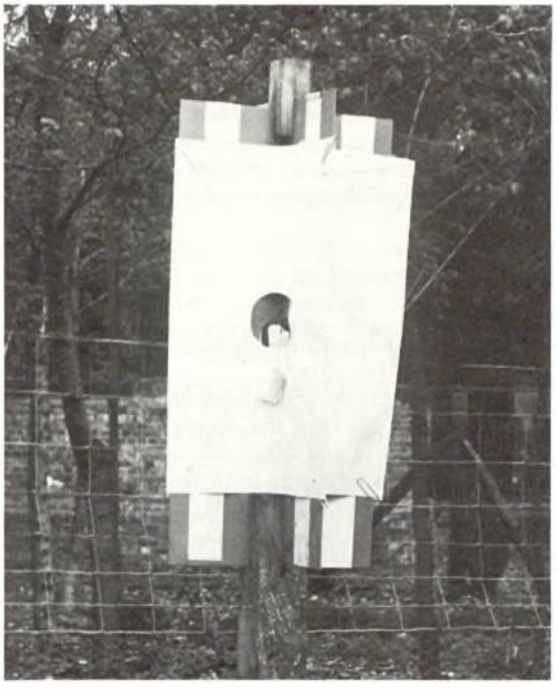

Plate 5 As Plate 4, with sticky white covers

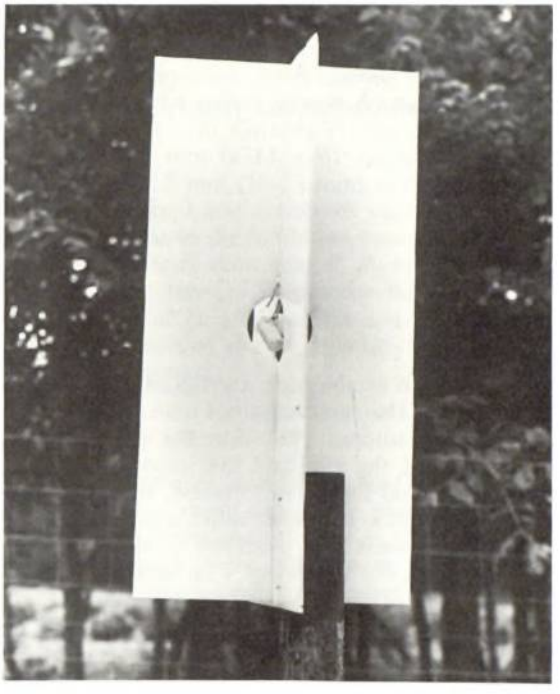

Plate 6 Disposable white PVC vertical fin trap

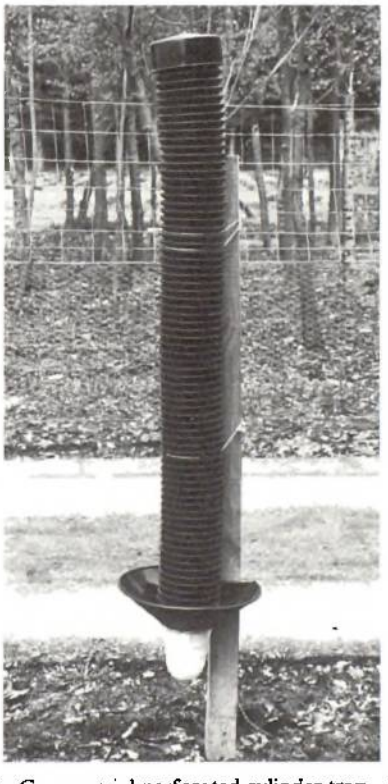

Plate 7 Commercial perforated cylinder trap – "Icopal"

PAPER 13

Pseudomonas fluorescens and *Ceratocystis ulmi* in wych elm

A. YDE-ANDERSEN
The Danish Plant Health Board

Introduction

With a view to establishing a prognosis for Dutch elm disease in Denmark enquiries were made during summer and autumn 1981 into the geographical distribution of the elm and the incidence of elm bark beetles and Dutch elm disease. In the course of the investigation a bacteria which seems to impede Dutch elm disease was observed in the wood of diseased elms; the following is an account of this phenomenon.

Material

In connection with the investigation of the incidence of elm bark beetles, 103 adult specimens of *Scolytus laevis* Chap. were taken from the bark of elms infected by *Ceratocystis ulmi* (Buism.) C. Moreau.

In two characteristic areas of attack, located respectively in Lolland-Falster and in Central Jutland, totalling about 800 km², samples showing characteristic discolouration of the xylem were collected from 42 wych elms which had been infected by the action of disease-carrying beetles; these elms were distributed in 20 localities.

Technique

From each wood sample at least three chips were taken and placed on malt extract agar, at least three chips were placed on selective medium (Brasier, 1981), and larger pieces of wood were placed in a moist chamber. The collected beetles were placed on selective medium.

After incubation at room temperature for 8 days, or in a moist chamber for 3 to 4 weeks, the samples were examined for the occurrence of micro-organisms. This assessment concentrated on *C. ulmi*, *Verticillium albo-atrum* Reinike et Berth., *Phomopsis oblonga* (Desm.) Trav. and bacteria; the occurrence of other micro-organisms was registered as contamination in the widest sense. During the examination of wood pieces incubated in moist chambers, records were made only of the occurrence of *C. ulmi* coremia and of bacteria.

In the investigation of elm bark beetles distinction was made only between the occurrence of *C. ulmi* mycelium, bacteria and other micro-organisms. The occurrence of bacteria was recorded due to the fact that in 1980 as well as in 1981, in cultures from discoloured xylem, bacteria occurred with remarkable frequency either alone or together with *C. ulmi* mycelium.

Results

On examination of the 103 elm bark beetles which had been removed singly from the bark of elms attacked by *C. ulmi* and placed on selective medium, the mycelium of the fungus was observed either alone or together with a slight bacterial flora around 23 beetles, while around 55 beetles only a slight bacterial flora could be observed. A single bacterial species was dominant.

Laboratory tests of the samples from the 42 wych elms in which discolouration of the xylem indicated attacks of *C. ulmi* had the following results:

On malt extract agar, *C. ulmi* mycelium was observed around all the chips from six elms. Around the chips from the remaining 36 elms only a heavy bacterial flora was observed.

On selective medium, *C. ulmi* mycelium together with a slight bacterial flora was observed around all the chips from a further 11 elms (giving a total of 17 elms). Around the chips from the remaining 25 elms only a slight bacterial flora was observed.

In moist chambers, *C. ulmi* coremia were observed on wood pieces from 27 elms (that is, all the elms from which *C. ulmi* mycelium had developed on selective medium and from a further ten).

In the cases mentioned, an exudation of bacteria from the discoloured xylem was often observed together with the *C. ulmi* coremia. The samples from the remaining 15 elms showed only bacterial exudation. The same bacterial species was dominant here. This was isolated and identified as *Pseudomonas fluorescens* (Trevisan) Migula.

Pseudomonas fluorescens

This is a rodshaped gram-negative bacterium which can be divided into four biotypes (Buchanan and Gibbons, 1974). It is common on organic material in nature and shows antagonism towards many bacterial species and also towards some micro-fungi (Waksman, 1952), but it is not pathogenic to higher plants.

Bacteria have been found previously in discoloured elm wood. In the Netherlands *Pseudomonas lignicola* Westerd. et Buism. has been detected in sharply defined, dark-coloured streaks in elm wood (Westerdijk and Buisman, 1929); the description of the species is however incomplete and no authentic culture exists (Buchanan and Gibbons, 1974). In the United States *P. fluorescens* has been found in elm wet wood (Murdoch and Campana, 1981).

In Canada a non-identified bacterium was isolated from elm wood, which *in vitro* showed antagonism towards *C. ulmi* (Pomerleau and Lechevalier, 1947). In the United States, 369 twigs from apparently healthy *Ulmus americana* L. were examined, and in 258 of them the wood appeared to contain micro-organisms, which were isolated. Of the total of 196 isolates of fungi, none was antagonistic towards *C. ulmi*, whereas among the total of 164 isolates of bacteria three were found to be antagonistic *in vitro* towards this fungus; the bacteria were not identified (Jewell and Campana, 1968). Finally, in Canada 18 elms with larval galleries chewed by *Hylurgopinus rufipes* Eichh. were examined. From 893 galleries a total of 695 isolates of both fungi and bacteria were collected, of which only 18 were *C. ulmi*. About fifteen per cent of the remaining isolates, which were not identified, inhibited the growth of *C. ulmi in vitro* (Stillwell, 1977).

It was therefore decided to investigate (in the first place *in vitro*) the behaviour of *P. fluorescens* and *C. ulmi* towards each other.

First, three strokes of a conidial suspension of the aggressive strain of *C. ulmi* were applied to ten petri dishes containing malt extract agar, followed immediately afterwards with a stroke of bacterial suspension. After three days incubation at room temperature the conidia within an average distance of 2 mm from the stroke had not germinated, and in the zone between 2 and 15 mm from the bacteria germination and mycelial growth were inhibited, whilst at a further distance copious mycelial growth was observed.

Next, ten petri dishes containing malt extract agar were inoculated with *C. ulmi* mycelium (aggressive strain), and after six days, during which time the mycelium had reached an average radius of 19.9 mm (18–24 mm), *P. fluorescens* suspension was inoculated into previously placed glass rings in the opposite side of each petri dish. After another 6 days at room temperature the radial growth of the mycelia was measured. Growth towards *P. fluorescens* was on average 10.9 mm (8–13 mm) against an average growth of 18.2 mm (15–23 mm) in the absence of the bacterium. Later numerous coremia appeared on the mycelia in the inhibition zone.

Thus, the tests show that *P. fluorescens* on malt extract agar secretes substances that inhibit the germination of *C. ulmi* conidia and the growth of the mycelium, and that it promotes the formation of *C. ulmi* coremia.

Discussion

The investigations thus show that specimens of *S. laevis* which have hatched in elms attacked by *C. ulmi* sometimes carry spores of this fungus, but that they much more often carry *P. fluorescens*. The use of selective culture medium and the heavy growth of *P. fluorescens* prevented the observation of other micro-organisms which beetles undoubtedly carry.

The demonstration of *C. ulmi* as the only micro-organism in the discoloured xylem of six elms may be explained as a consequence of the elm bark beetles carrying only spores of this fungus. However, it may also be considered the result of the introduction of *C. ulmi* together with one or more other micro-organisms, but where for unknown reasons *C. ulmi* has become completely dominant.

The demonstration of *C. ulmi* together with *P. fluorescens* in the discoloured xylem of 21 elms may be taken as an indication of a simultaneous infection of the feeding wound with both micro-organisms, but may also be considered the result of infection of different wounds on the same twig or on different twigs, each with its own micro-organism. The investigations do not allow final conclusions to be drawn.

However, the finding that *P. fluorescens* was the only micro-organism in 15 elms may indicate that, under certain circumstances, this bacterium has been capable of superseding *C. ulmi*; this impression is strengthened by the antagonism towards *C. ulmi* observed from *P. fluorescens* on malt extract agar.

Acknowledgement

The identification of *P. fluorescens* was courteously made by Dr. J. F. Bradbury, of the Commonwealth Mycological Institute, Kew, Richmond, Surrey, TW9 3AF, U.K.

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PAPER 14

Biological control of Dutch elm disease by *Pseudomonas* species

R. J. SCHEFFER

Phytopathological Laboratory 'Willie Commelin Scholten', Javalaan 20, 3742 CP Baarn, The Netherlands

Introduction

For the control of Dutch elm disease several strategies have been employed (Sinclair, 1978). Of these strategies, quarantines can theoretically exclude Dutch elm disease from an area, provided that there are elm-free natural barriers. Once Dutch elm disease is established, eradication of the centre of disease is a virtually impossible way of preventing spread to other areas. The other control strategies all reduce the infection rate: sanitation and/or spraying with insecticides for suppression of vector populations; preventing root graft transmission; the use of systemic fungicides, and planting of elms with a high degree of resistance. With the exception of the planting of resistant elms (if available) these control strategies have one important thing in common: they are expensive.

Both the havoc made by Dutch elm disease and the cost of control may explain the constant interest in alternative strategies of which biological control of both beetle and fungus have recently received considerable attention.

Phomopsis oblonga for biological control of elm bark beetles

A natural biological control of elm bark beetle populations by the fungus *Phomopsis oblonga* was observed in Wales (Webber, 1981). This fungus apparently makes dying or recently dead elms unattractive for breeding by elm bark beetles. If their eggs were laid in *Phomopsis*-colonized trees only a few developed into a new generation of beetles. Massive colonization of elm bark by *Phomopsis* has mainly been observed in *Ulmus glabra*. However, it is not yet clear if manipulation of *Phomopsis* can result in the extension of its beetle-controlling attribute to other elms or areas.

Bacterial antagonism of Ceratocystis ulmi

In vitro antagonism against *Ceratocystis ulmi* (Buisman) C. Moreau (=*Ophiostoma ulmi* (Buisman) Nannf.), the fungus causing Dutch elm disease, has been observed already by Hendrickx (1937). He isolated a bacterium from an elm branch infected by *C. ulmi* which inhibited pure cultures of that fungus on malt extract agar. Some evidence was presented that the bacterium was *Pseudomonas lignicola*, also isolated from elm by Westerdijk and Buisman in 1929.

Other examples of bacterial antagonism against *C. ulmi in vitro* were presented by Pomerleau and Lechevalier (1947), Holmes (1954), Jewell (1967) and Semer (1978). It could not be demonstrated, however, that bacteria were able to suppress *C. ulmi* within the living tree.

In 1981, G. A. Strobel and D. F. Myers presented papers at the annual meeting of the Pacific Division of the American Phytopathological Society in which they announced a remarkable success in suppressing Dutch elm disease by injecting *Pseudomonas syringae* suspensions into diseased trees (Strobel and Myers, 1981; Myers and Strobel, 1981).

Laboratory investigations with Pseudomonas bacteria

As a result of personal discussions with Strobel, we were able to plan experiments at Baarn early in 1981 in order to test under Dutch conditions the bacterial strain used by the group of Strobel. In addition, a screening programme was carried out for determining antagonistic characteristics towards *C. ulmi* on agar media of a selected group of bacteria. These bacteria, all members of the genus *Pseudomonas*, were chosen from the collection of F. P. Geels and B. Schippers at our laboratory on the basis of already known antagonistic characteristics towards a series of fungi and bacteria, both plant pathogens and saprophytes. From this collection three bacterial isolates (coded WCS 085, WCS 361 and WCS 374) were finally chosen for field experiments because of their outstanding antagonistic properties *in vitro*.

Field trials with Pseudomonas bacteria

At the tree nursery of the State Forestry Service at Baarn 180 'Commelin' elms (*Ulmus* x *hollandica*

'Commelin') with an average diameter (DBH) of 6 cm and a height of approximately 6 m could be used. In all experiments, trunks of the trees were inoculated with a highly aggressive isolate of *C. ulmi* (North American (NAN) race). For bacterial treatments one of the three *Pseudomonas* isolates selected from our collection (WCS 085, WCS 361 or WCS 374) or *Pseudomonas syringae* M 27+, the one used by Strobel and co-workers, was used. Bacteria were applied in two different ways: by low-pressure injection of a suspension of 10^8 cells per ml or by injection with a 1 cm wide gouge of small quantities of a suspension in its stationary growing phase. For low-pressure injection (1½ bar) standard nylon injection tees were used for which holes of 8 mm diameter had to be drilled into the trunk of the trees.

Two types of experiments were performed: a series of curative experiments where the trees were inoculated with *C. ulmi* first and with one of the bacterial isolates approximately one week later, and a series of preventive experiments where the trees were treated with bacteria first and then, after one to four weeks, inoculated with *C. ulmi*.

Results

In the series of curative experiments all groups of trees developed serious symptoms. After pressure injection only the group of trees treated with WCS 361 showed somewhat less symptoms than the controls. From the group of trees treated with a gouge only the group treated with WCS 374 showed significantly less symptoms compared with the controls. It must be emphasized, however, that the trees were treated with bacteria at a moment when *C. ulmi* was already widespread throughout the trees.

In the series of preventive experiments also only one of the pressure-injected groups of trees showed less symptoms than the controls (again the group treated with WCS 361). But all four groups of trees treated with a gouge showed significantly less symptoms compared with the controls. Many trees even showed no symptoms at all at the end of the growing season.

Future research and prospects

Because of these encouraging results experiments in conjunction with the Dutch Plant Protection Service and the State Forestry Service are planned for the 1982 growing season. Bacteria will be introduced to large groups of elms as a preventive treatment, using sophisticated 'home-made' gouge pistols by which one person can administer the injections. In some experiments inoculations with *C. ulmi* will be carried out in the crowns of the trees, in others the number of naturally infected trees showing Dutch elm disease symptoms will be compared with control groups.

Besides initiating these new experiments the effects of the treatments of those started in 1981 will also be followed; both the experiment with 180 'Commelin' elms discussed above, and the curative experiment. For this latter experiment, naturally infected elms in four areas of the Netherlands were treated by pressure injection of trunk or root flares. However, differences between injected and non-injected trees were not seen to be significant at the end of the 1981 growing season.

Hopefully the practical value of this biological control method can be evaluated during 1982 on the basis of these experiments. If judged to be practically applicable, then large scale treatment with bacteria may considerably reduce infection rates. In that case biological treatment could become an important factor in the control of Dutch elm disease.

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PAPER 15

Host-parasite interactions in Dutch elm disease

D. M. ELGERSMA

Phytopathological Laboratory 'Willie Commelin Scholten', Javalaan 20, 3742 CP Baarn, The Netherlands

Introduction

The highly destructive Dutch elm disease caused by *Ceratocystis ulmi* (Buisman) C. Moreau) (= *Ophiostoma ulmi* (Buisman) Nannf.) has been known now for more than 60 years. Since the early 1920s plant pathologists have studied this disease and an increasing amount of information has been gathered (Sinclair and Campana, 1978; MacDonald and Hindal, 1981. Host-parasite interactions in wilt diseases such as Dutch elm disease are complex. Effects induced directly or indirectly by the pathogen on the host are difficult to separate and their relative importance in the disease syndrome is difficult to measure.

After infection by *C. ulmi* has taken place the tree shows typical leaf symptoms, such as yellowing, wilting and necrosis, which finally result in death of the tree. The rapidity of symptom development depends on many factors such as the genetic make up, vigour and age of the tree. Also nutrient status, soil condition, and temperature play a role and the time of year that infection takes place is very important. On the other hand virulence of the pathogen is also important. All these factors can influence the prognosis of a tree after infection by the Dutch elm disease pathogen.

Pathogenesis

Invasion of the host by Ceratocystis ulmi

The important question is however, how does it happen that a tree wilts and dies? The fungus once introduced into the vascular system grows and is transported through the whole tree. This is essential for disease development. Interaction of fungal metabolites with the physiology of the tree leads to the wilt syndrome. Initial growth of the pathogen can be accomplished by the available nutrients in the xylem sap (Elgersma, 1969; Singh and Smalley, 1969). Production of cell wall degrading enzymes by the pathogen can provide the fungus with nutrients directly by releasing monosaccharides from cell wall polymers or by causing death of parenchyma cells (Basham and Bateman, 1975) the contents of which then form a potential nutrient source. These enzymes may be involved in the penetration of pits of vessel walls or endplates enabling the spread of the fungus through the plant. Partially hydrolysed cell wall polysaccharides and cell contents of parenchyma cells in the vessel lumina may impede the water transport and so influence the total water economy of the tree.

Cell wall degradation

Recently Scheffer and Elgersma (1982) have studied xylem vessel wall degradation in American elms after infection of the tree with either an aggressive or a non-aggressive isolate of *C. ulmi* by scanning electron microscopy.

It appeared that vessel walls were much more affected by the aggressive isolate than by the non-aggressive one. Erosion of vessel walls was not necessarily restricted to the wall underneath hyphae or conidia, but can also occur where there is no direct contact with the fungus. Observation of hyphae and conidia at least in this rather late stage of disease, was much more difficult in elm wood infected by the non-aggressive than by the aggressive one. This is in agreement with the results obtained by Elgersma and Heybroek (1979). They showed that survival of an aggressive isolate was better than that of a non-aggressive one in susceptible as well as in resistant trees. In accordance with earlier observations (Miller and Elgersma, 1976; Elgersma and Steerenberg, 1978) no evidence was found indicating that the fungus invades tissue surrounding the xylem vessels. Hyphal penetration of pits only was observed.

The results as described above are also in close harmony with the results obtained by Svaldi and Elgersma (1982). They studied the production of cell wall degrading enzymes of aggressive and non-aggressive isolates *in vitro*. Glycosidase and exo-glycanase activity of several aggressive and non-aggressive isolates was determined. The cell wall degrading enzymes were produced by the fungus

grown on pulverized elm wood. Enzyme preparation from aggressive isolates released more arabinose and xylose from cell walls of elm wood than those from non-aggressive isolates. Significantly more rhamnose too, was released by aggressive isolates although some of the non-aggressive isolates released comparable amounts. No correlation, however, was found between polygalacturonase and cellulase production and aggressiveness (Elgersma, 1976). All these results strongly indicate that cell wall degrading enzymes are involved in pathogenesis.

Other mechanisms of vessel disruption

These enzymes together with production of growth substances by the pathogen or host can induce tyloses (Beckman, 1971), which may play a role in blocking the water transport in the vessels. On the other hand gelation of primary cell walls by acidification can also be responsible for impeding waterflow (Van der Molen *et al.*, 1977; Beckman and Talboys, 1981). As indicated during this seminar, toxins undoubtedly play a role in disease development (Scheffer, 1983). Macromolecular toxins such as glycopeptides which have recently been detected in infected xylem sap (Scheffer and Elgersma, 1981), may plug the ultrapores of pit membranes of vessels and so substantially hamper water movement in the tree (Van Alfen and Turner, 1975; Van Alfen and MacHardy, 1978).

Mechanisms of resistance

What can the tree do against all these effects so detrimental to its existence, in order to survive? The prognosis depends largely on its genetic make up: if it has enough genes for resistance it may survive, if not it mostly dies. Resistance to Dutch elm disease is polygenetically controlled (Heybroek, 1970; Lester and Smalley, 1972) and probably quantitative in nature.

Phytoalexins

An effective method of defence would be to stop the fungus from growing or even to kill it by host production of fungitoxic compounds released into the vessel lumina. Indeed, substances called phytoalexins are produced but we have not found a more rapid accumulation of these substances in the more resistant trees (Overeem and Elgersma, 1970; Elgersma and Overeem, 1971). This does not imply that these substances do not play a role in resistance. Together with other mechanisms such as tylose formation they may be very effective.

Tyloses

These, if formed rapidly enough may seal off the vessels in advance of the fungus and by that means localize the infection (Elgersma, 1973).

Barrier zones

Shigo and Tippet (1981) studied Dutch elm disease from the point of view of the compartmentalization of decay in trees (Shigo, 1979). They observed in infected American elms barrier zones of starch-containing parenchyma and swollen ray parenchyma. Such barrier zones act as a tangential shield between infected xylem and healthy cambium allowing the regeneration of healthy tissue. If rates of compartmentalization and regeneration exceed that of infection, the tree will stay alive. If the infection rate exceeds the rate of compartmentalization, the tree will die.

Barrier zone formation probably also requires a great amount of energy. Depletion of the stored nutrients in springtime by leaf development, new growth ring development and seed production, means that barrier zone formation may be not so effective as no new carbohydrate is formed following wilting of leaves. The cambium may be killed and the tree dies. It seems obvious that barrier zone formation can play a role in defence. How important this mechanism is and how serious the effect of nutrient depletion can be in disease development, certainly needs more investigation.

Anatomical characteristics

Besides physiological defence mechanisms anatomical factors can play a role in resistance. Summerwood for instance is characterized by smaller, more scattered and less contiguous vessels than springwood, allowing more time for defence mechanisms to operate and confine the pathogen. Sufficient non-infected vessels are thus left for water transport (Pope, 1943). In Dutch elms as well as in American elms it was shown that the average diameter of xylem vessels was smaller in resistant elms than in susceptible ones (Elgersma, 1970; Sinclair *et al.*, 1975). Resistant elms also have smaller groups of clustered vessels separated by parenchyma tissue, which limits lateral spread of infection in these trees (McNabb *et al.*, 1970). It is obvious that these anatomical features, in addition to the observed faster tylose formation in resistant elms which may seal off the vessels before conidia have spread, can be effective mechanisms in localizing the infection (Elgersma, 1973).

Pathogenic advantage of the aggressive strain of Ceratocystis ulmi

Aggressive isolates, however, can more or less overcome these mechanisms (Miller and Elgersma, 1976). When a mixture of aggressive and non-aggressive isolates was used for inoculation of trees resistant to the non-aggressive strain, spreading of the non-aggressive isolate was limited and survival of the aggressive isolate was favoured (Elgersma and Heybroek, 1979). As already mentioned, a higher production of cell wall degrading enzymes by the aggressive strain may play a role in this phenomenon. When a mixture of both strains was used disease symptom expression was significantly reduced in a resistant clone, compared to that seen after inoculation with the aggressive strain alone. It appears that the non-aggressive strain triggers a mechanism which counteracts at least partially the induction or expression of disease symptoms provoked by the aggressive strain alone (Scheffer *et al.*, 1980).

Conclusion

There is much evidence for the proposed mechanisms of resistance described above, but no absolute proof has been obtained.

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PAPER 16

Toxins in Dutch elm disease

R. J. SCHEFFER

Phytopathological Laboratory 'Willie Commelin Scholten' Javalaan 20, 3742 CP Baarn, The Netherlands

Introduction

Toxin research constantly faces problems with respect to detection and purification of minute quantities of the sometimes unstable compounds. Methodology in this field is still under development but great (although often underestimated) progress has been made during the last decade. Gas-liquid chromatography, sometimes coupled with mass-spectrometry, high performance liquid chromatography, ultra- and gel filtration, affinity chromatography and various electrochemical separation methods were developed or greatly improved. Advances in immunological detection methods were, if possible, even greater.

The impact of these technical advances on toxin research has been tremendous. Many toxins have been studied, resulting in greater insight into the role of toxins in pathogenesis. The importance of toxins in Dutch elm disease pathogenesis is one of the more carefully studied cases in toxin research. In this paper, the present status of this work will be discussed.

Toxins produced by Ceratocystis ulmi

Ceratocystis ulmi (Buisman) C. Moreau (*Ophiostoma ulmi* (Buisman) Nannf.), the causal organism of Dutch elm disease, can easily be grown on synthetic media. Over the years several phytotoxic compounds have been isolated and purified from shake cultures, with varying success. These phytotoxic compounds can be split up into four groups: phenolics, polysaccharides, a protein named cerato-ulmin and a glycopeptide. These four groups will be discussed here*.

Phenolics

The phenolic metabolites of *C. ulmi*, investigated by Claydon *et al.* (1974, 1980) express phytotoxicity in elm shoots. Attempts to reproduce phytotoxic effects by injecting these compounds into stems of four-year-old rooted elm saplings were unsuccessful, however, and no evidence was obtained pertaining to their *in vivo* formation.

Polysaccharides

A polysaccharide fraction can be precipitated from culture filtrates with 70 per cent ethanol. In 1947 Dimond observed some effects of this material on tomato and elm cuttings, probably due to impediment of the water flow. The importance of these polysaccharides *in vivo* is questionable. Apparently the viscosity of the xylem fluid is not affected after infection of the tree by *C. ulmi* (Dimond and Husain, 1958). Thus, polysaccharide production should be low in comparison to production in shake cultures where viscosity rises rapidly due to polysaccharide production. Both Feldman *et al.* (1950) and Rebel (1969) considered this polysaccharide fraction to be of minor or no importance, as concentrations necessary to induce wilting were even higher than that occurring in shake cultures.

Cerato-ulmin

A small protein possessing phytotoxic properties was isolated by Takai and co-workers and named 'cerato-ulmin' (Richards and Takai, 1973; Takai, 1974; Takai and Richards, 1978). Production of cerato-ulmin in shake cultures is reported to be correlated with aggressiveness of the fungal strain in many cases (Takai, 1974, 1980) but exceptions have been noted (Barret and Skidmore, 1975; Takai, 1980). Administered to elm cuttings, cerato-ulmin induces wilting, chlorosis and necrosis (Takai, 1974). Wilting could be induced by placing the shoots in a solution containing 7.5 µg cerato-ulmin/millilitre of water. Extremely low concentrations, as low as 2 ng/ml, are claimed to be toxic also (Takai *et al.*, 1979).

Takai and co-workers suggest that cerato-ulmin does not induce wilt in elms by blocking the vessels,

* Part of this discussion was derived from a paper presented at the Symposium and Workshop on Dutch elm disease, Winnipeg, Canada, October 1981.

as concentrations of cerato-ulmin necessary to induce symptoms in shoots are probably too low to physically interfere with the water flow. Instead, they propose that cerato-ulmin triggers an unknown host response.

Russo *et al.* (1981), however, found that the surface tension of a cerato-ulmin solution was still affected at a concentration of 30 ng/ml although only a fraction of a monolayer could be formed at that concentration even if all the cerato-ulmin rose to the air-water surface. The solubility in water also proved to be extremely low, less than 10 μg/ml. When the concentration increases turbidity is observed due to small air bubbles coated with cerato-ulmin. These surface-active properties of cerato-ulmin mean that recognition by the host may not be necessary but that simple plugging or waterproofing of the pit membranes may account for the results of bio-assays.

Antiserum against cerato-ulmin was produced (Krywienczyk *et al.*, 1979) and double diffusion tests were performed against extracts obtained from discoloured xylem wood from elms infected by *C. ulmi*. Preliminary results indicate that cerato-ulmin may be present in such extracts (Takai *et al.*, 1981).

Glycopeptide

A partially purified glycopeptide fraction was obtained by Salemink *et al.* (1965) and later by Rebel (1969). Using affinity chromatography, Strobel *et al.* (1978) obtained a polydisperse glycopeptide fraction which was apparently free from contaminants. Structural analysis by Nordin and Strobel (1981) revealed that the material is a polydisperse peptidorhamnomannan with a molecular weight range of approximately 105,000 to 120,000. Phytotoxicity of the glycopeptide fraction in elm shoots has been reported by Salemink *et al.* (1965), Rebel (1969), Strobel *et al.* (1978) and in more detail by Van Alfen and Turner (1975). The latter found that as little as 4 μg of glycopeptide or high molecular weight dextran measurably decreased the stem and petiole conductance in elms. Their conclusion is that physical plugging of the pit membranes and possible cavitation is the most likely mode of action of the glycopeptide. In a later study, Van Alfen and Allard-Turner (1979) showed that even lower amounts of dextrans could seriously reduce vascular conductance.

Investigations into the glycopeptide toxin produced by Ceratocystis ulmi

Within the framework of the EEC Project on Dutch elm disease a study was initiated in order to obtain data on *in vitro* and *in vivo* production of the glycopeptide(s) produced by *C. ulmi*. Our preliminary results indicate that there are no important differences in total peptidorhamnomannan production in shake cultures between several aggressive and non-aggressive isolates of *C. ulmi*. There may be structural differences, however.

Using the enzyme-linked immunospecific assay (ELISA) we showed that in the susceptible elm, *Ulmus hollandica* 'Belgica', accumulation of the glycopeptide began two weeks after inoculation with the aggressive isolate H6 of *C. ulmi* and increased to approximately 5 μg/ml wood sap four weeks after inoculation (Scheffer and Elgersma, 1981a; see Figure 1).

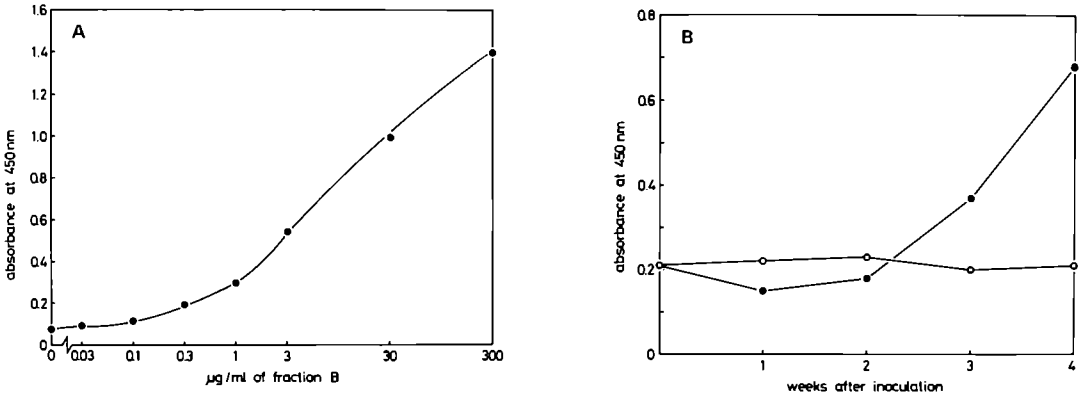

Figure 1a: Absorbance of different concentrations of glycopeptide Fraction B of *C. ulmi* measured after 1 h of incubation for ELISA.

Figure 1b: Glycopeptide production in susceptible *U. hollandica* 'Belgica' inoculated with *C. ulmi*, as measured by absorbance after 1 h of incubation for ELISA. (●) Infected plants; (○) healthy control plants. Each datum point is the average of five replicates. (From: Scheffer and Elgersma, 1981a).

Since the wood sap was collected by means of a hydraulic press, the sap was diluted by fluid from other tissues. In addition, part of the glycopeptide might have been trapped in the ultrapores of pit membranes or might possibly have reacted with host cell wall polysaccharides, as recently was suggested by Nordin and Strobel (1981), and therefore was not recovered. Thus, the concentration *in situ* must have been higher and beyond all doubt was high enough to expect phytotoxic effects.

In the 1980 field trials the moderately resistant clone '390' was inoculated with the aggressive isolate H6 of *C. ulmi*. The trees showed severe symptoms with increasing time after inoculation and the fungus spreads readily throughout the tree (Elgersma and Heybroek, 1979). However, it was found that there were no differences in ELISA results between inoculated and control trees. Pure glycopeptide, added to these wood saps proved to be quantitatively untraceable with increasing time after inoculation; a phenomenon which was not observed in the experiments with the susceptible elm mentioned above. Apparently some interaction between wood sap and glycopeptide took place during the ELISA (Scheffer and Elgersma, 1981b). High molecular fractions of the wood saps, obtained by gel filtration on Sephacryl S 300 (Pharmacia) did not exhibit this yet unexplained interaction. Also, it was possible to detect glycopeptide in high molecular fractions of wood saps from elms of the clone '390' inoculated with the aggressive isolate H6 of *C. ulmi*.

Moreover, the following results of our *in vivo* experiments should be mentioned here: the highly resistant *Ulmus pumila* and the clones 'Lobel' and '519' did not show external symptoms after inoculation with an aggressive isolate (H6) of *C. ulmi*. Using ELISA no glycopeptide could be detected.

The susceptible *Ulmus hollandica* 'Belgica', *U. americana* and *U. glabra*, however, developed heavy symptoms of which the first visible signs appeared after approximately one week (1981 field trials). Low levels of glycopeptide were detectable on the first sample date following the appearance of external symptoms. Later, glycopeptide increased to levels comparable to that reported earlier for *U. hollandica* 'Belgica' (Scheffer and Elgersma, 1981a), that is: between one and ten µg/ml.

Conclusions

From the phytotoxic compounds reviewed here only two seem to be candidates for a serious role in pathogenesis: cerato-ulmin and the glycopeptide. Only for the latter compound is its presence in diseased hosts satisfactorily demonstrated. The glycopeptide is the first compound, therefore, that should be regarded as a vivotoxin or a vivoaggressin (Graniti, 1972; Scheffer, 1982).

Generally, in the wilt diseases most evidence points towards impediment of the water flow as the cause of external symptom development (Dimond, 1970). For Dutch elm disease also many things point towards this mechanism: after infection by *C. ulmi* the water flow can be impeded by tyloses and gums (Van Alfen and MacHardy, 1978), by plugging of pit membranes by degradation products of host cell walls (Elgersma, 1976; Scheffer and Elgersma, 1982; Svaldi and Elgersma, 1982) and by toxins. But also, phenomena are observed after infection of an elm by *C. ulmi* which cannot be explained by this water flow impediment theory: for instance, increased resistance of roots to water flow (Roberts and Schreiber, 1977), increased respiration and increased electrolyte leakage (Landis and Hart, 1972).

It is obvious that toxins are involved in Dutch elm disease pathogenesis. But it is equally obvious that their role in the development of the complex Dutch elm disease syndrome is yet only partially understood.

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PAPER 17

Studies on *Ceratocystis ulmi* in Belgium

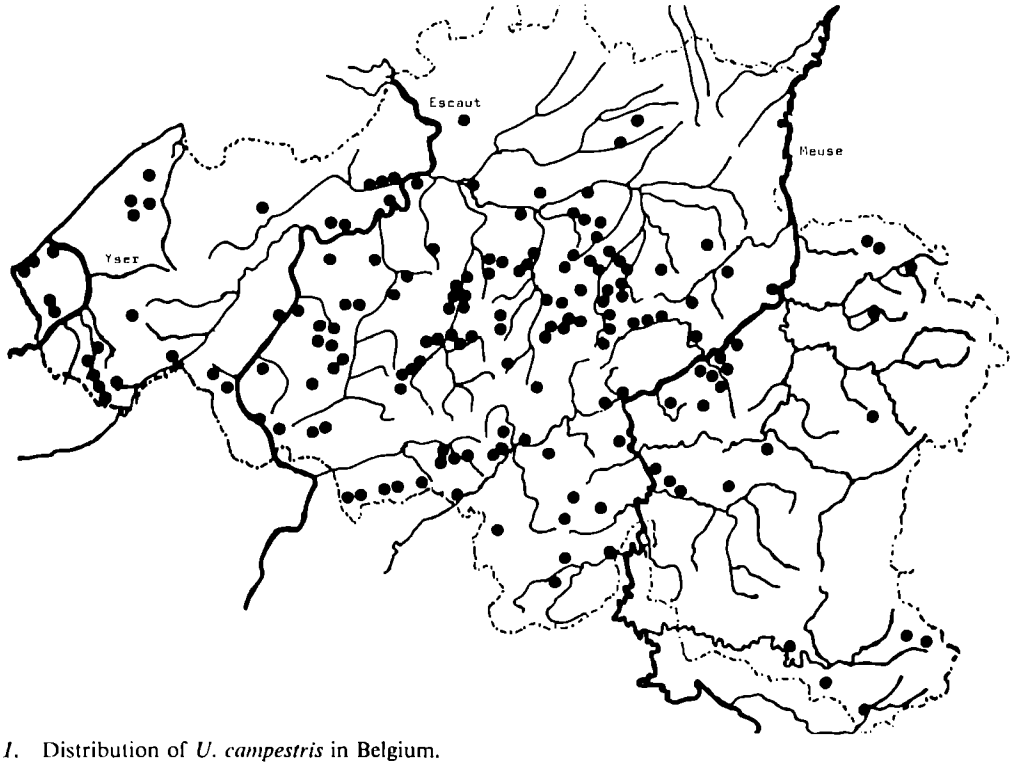
M. MEULEMANS and C. PARMENTIER

Laboratoire de Pathologie Végétale, Faculté des Sciences Agronomiques, Avenue Maréchal Juin, 13, B-5800 Gembloux, Belgium

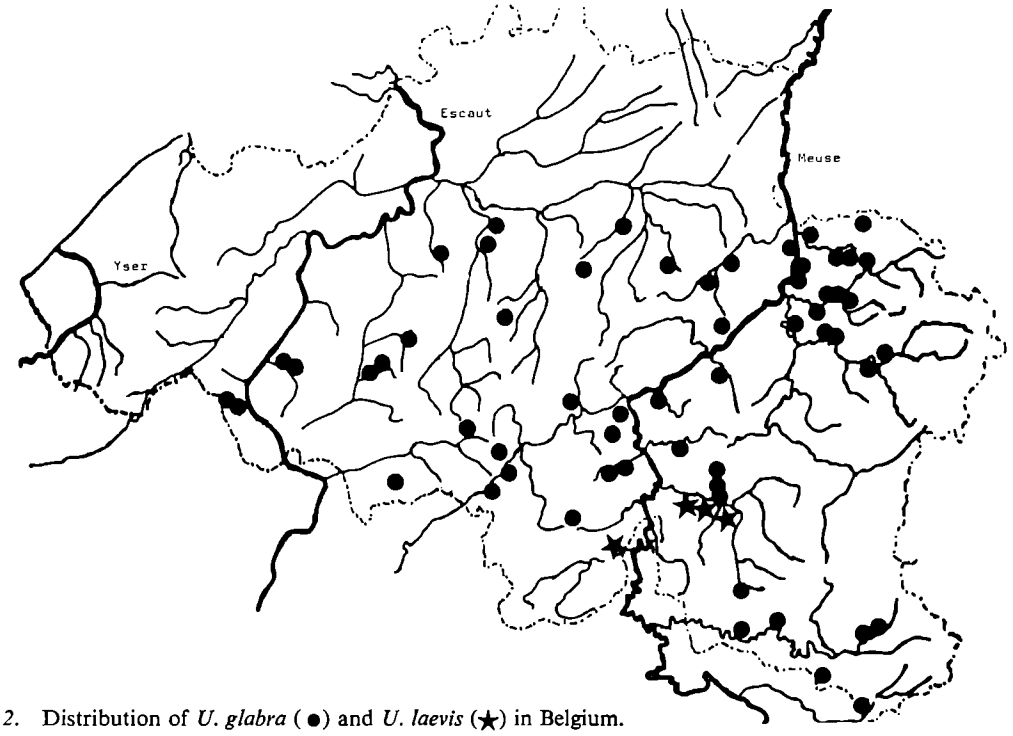
Introduction

Huberty (1904, 1905) reports that in the 16th century, Dodonee, a physician and botanist from the city of Malines, was struck by the importance of elms in our forests. In 1601, at the request of Sully, minister of the French King Henry IV, elms were planted along roads and public squares in France, to celebrate the birth of Louis XIII. Until the First World War, a number of cultivars belonging mainly to the species *Ulmus campestris* L. and *Ulmus glabra* Huds., were planted on a large scale, together with *U. x hollandica* Mill., a hybrid between these two species. Some clones were also selected in Belgium for example, *U. campestris* cv. 'Dampieri' (in 1853), *U. procera* cv. 'Louis van Houtte' (in 1880) and the well known *U. x hollandica* cv. 'Belgica' (in 1694). Some local varieties, such as the "Orme dur de Furnes", the "Orme à fines feuilles du pays d'Ypres" and the "Orme tortillard" were also highly praised.

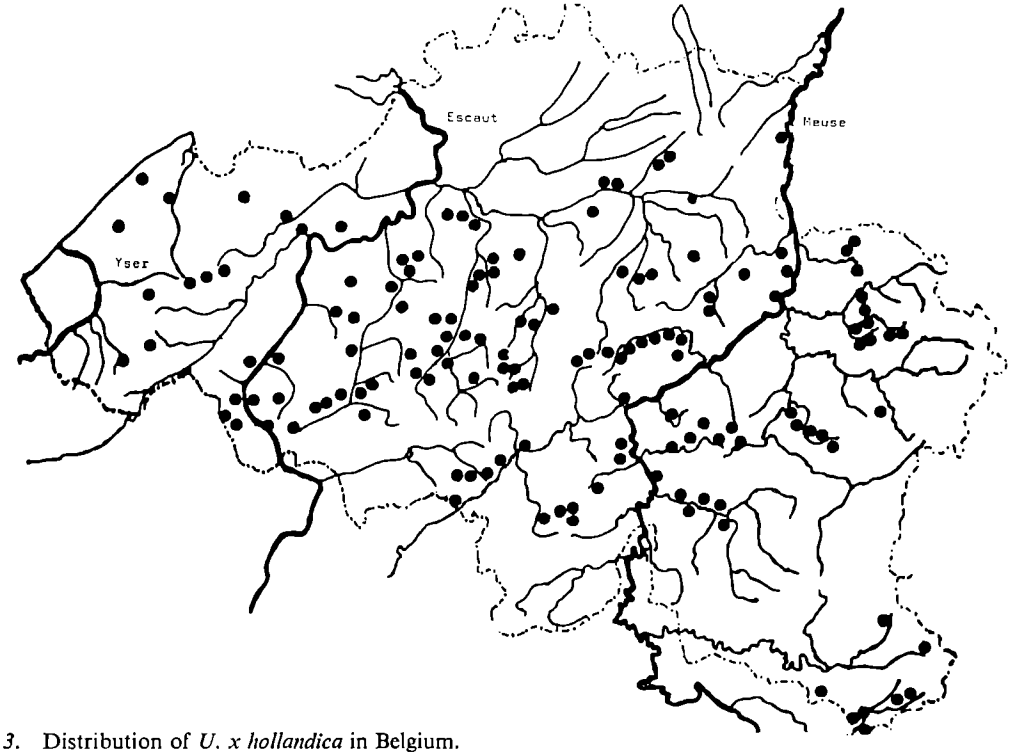
In Belgium, the first die-back of elms was observed in 1836, in the park of Brussels. Disease was also observed at the same place in 1896, and the elms of the "Place d'Armes" in Ghent were killed in 1885–1886. At this time, the die-back of elms was attributed to the elm bark beetle (Severin, 1906). After 1918, public opinion was shaken by the unprecedented death of elms. The Administration



Map 1. Distribution of *U. campestris* in Belgium.



Map 2. Distribution of *U. glabra* (●) and *U. laevis* (★) in Belgium.



Map 3. Distribution of *U. x hollandica* in Belgium.

Table 1. Healthy elm sites in the regions of Belgium (numbers and percentage).

Region / Elm species	Basse Belgique	Moyenne Belgique	Haute Belgique	For all areas
U. campestris	8 (11%)	6 (7.5%)	7 (18%)	21 (11%)
U. glabra	1 (5%)	3 (15%)	14 (28%)	18 (20%)
U. laevis	absent	absent	3 (75%)	3 (75%)
U. x hollandica	6 (10.5%)	3 (5%)	11 (18%)	20 (11%)
Not determined	absent	3 (14%)	6 (33%)	9 (15%)
Total species	15 (10%)	15 (9%)	41 (26%)	71 (14%)

of Forests (in 1919, 1925, 1928 and 1933) and Roads (in 1919) made inquiries to follow the spread of the disease. Judging by the descriptions of symptoms at that time, it seems that the damage was due indeed to the causal fungus of Dutch elm disease (DED). Most elms were lost between 1918 and 1940; from 1940 to 1970 the disease decreased progressively in Belgium. This paper will describe the observations on DED made here in recent years.

Epidemiology

Host

Three species of elms occur naturally in Belgium: *U. campestris* (European field elm), *U. glabra* (wych elm), and *U. laevis* Pall. (European white elm).

The European field elm is distributed throughout the country, with a higher density in "Moyenne Belgique"; wych elm is mainly found in "Haute Belgique" (maps 1 and 2). The European white elm is endemic in a few valleys (Molignee, Lesse and Viroin) (map 2). The *U. x hollandica* hybrid elm is present throughout the country (map 3).

In Belgium the elm occurs mostly as bushes (along roads or railways), medium height trees, and only rarely as trees of larger size.

Importance of Dutch elm disease

DED-free populations of elm are now few in number: 10 per cent of the locations are healthy in "Basse Belgique" or "Moyenne Belgique", while 26 per cent of elm locations are disease-free in "Haute Belgique". The difference may result from the lower density of elm populations and the less favourable climate for the elm bark beetle in the higher areas of the country. Out of 515 elm locations observed in Belgium, 444 are diseased (Table 1). Nevertheless, larger trees (diameter of more than 50 cm) are less frequently attacked than others (Table 2). Gremmen *et al.* (1976) observed the same phenomenon in the Netherlands.

Vectors of Dutch elm disease

The two elm bark beetle species which were most frequently observed during our investigations are *Scolytus scolytus* F. and *Scolytus multistriatus* Marsh.; the species *Hylesinus vittatus* Fabr. (whose galleries run across the grain), and other species of small bark beetles, are less common.

Table 2. Health in relation to size of elms in Chastre (Brabant).

Tree diameter	Elms (number and percentage)		Total
	Healthy	Diseased or dead	
More than 50 cm	889 (80%)	223 (20%)	1112
From 15 to 50 cm	684 (41%)	998 (59%)	1682
Less than 15 cm	248 (38%)	411 (62%)	659
Total	1821 (53%)	1632 (47%)	3453

The fungus

The development of a new outbreak of DED in Belgium in the 1970s gave rise to some concern (Veldeman, 1979; Mertens *et al.*, 1979). The first signs of the re-appearance of this disease in Belgium date back to 1972, when the Authorities of the city of Liège were concerned about elm deaths.

Our first investigations on DED started in 1978 (Meulemans and Semal, 1978) and since then more than 500 samples from diseased elms have been collected in the country (Meulemans *et al.*, 1979, 1981). Determination of growth-rate and culture morphology on 2 per cent malt extract agar (Oxoid) permitted the identification of two strains of *Ceratocystis ulmi*, aggressive and non-aggressive, as defined by Gibbs and Brasier (1973) and Brasier (1981).

Out of 500 isolates, 460 had the characteristics of the aggressive strain (fluffy type of colonies and high growth rate), and 40 were non-aggressive (waxy appearance of the colonies and slow growth rate). The growth rate distribution of the various isolates gives a Gaussian curve for the aggressive strain, whereas the growth rate distribution for the non-aggressive strain is very irregular in shape (Figure 1).

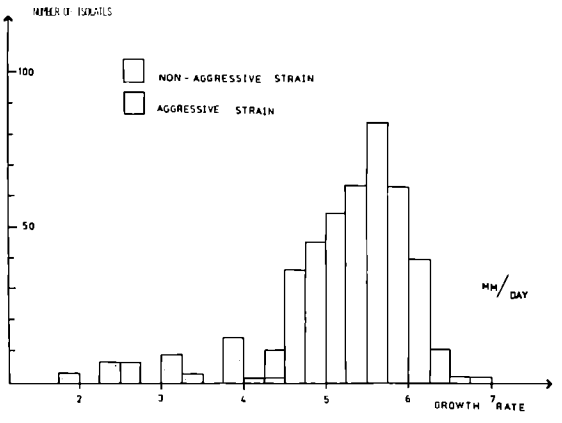


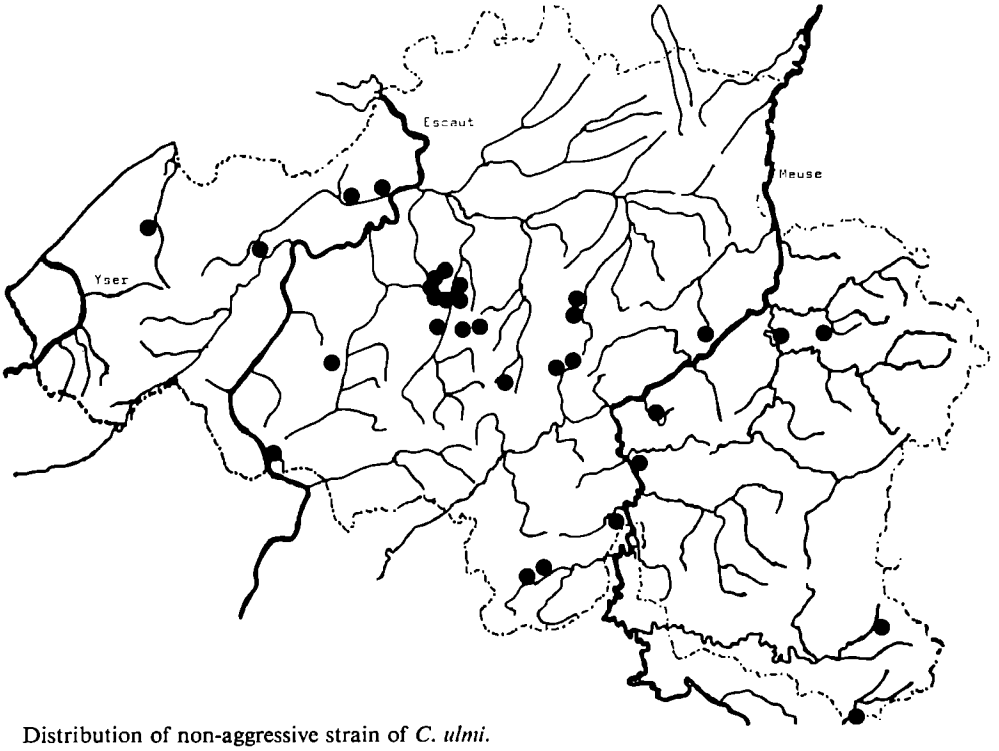
Figure 1. Histogram of the growth rate frequencies for aggressive and non-aggressive strains on malt extract agar.

The aggressive strains of *C. ulmi* fall into two distinct races, the Eurasian (EAN) and the North-American (NAN) races, on the basis of their reciprocal fertility (Brasier, 1979, 1981).

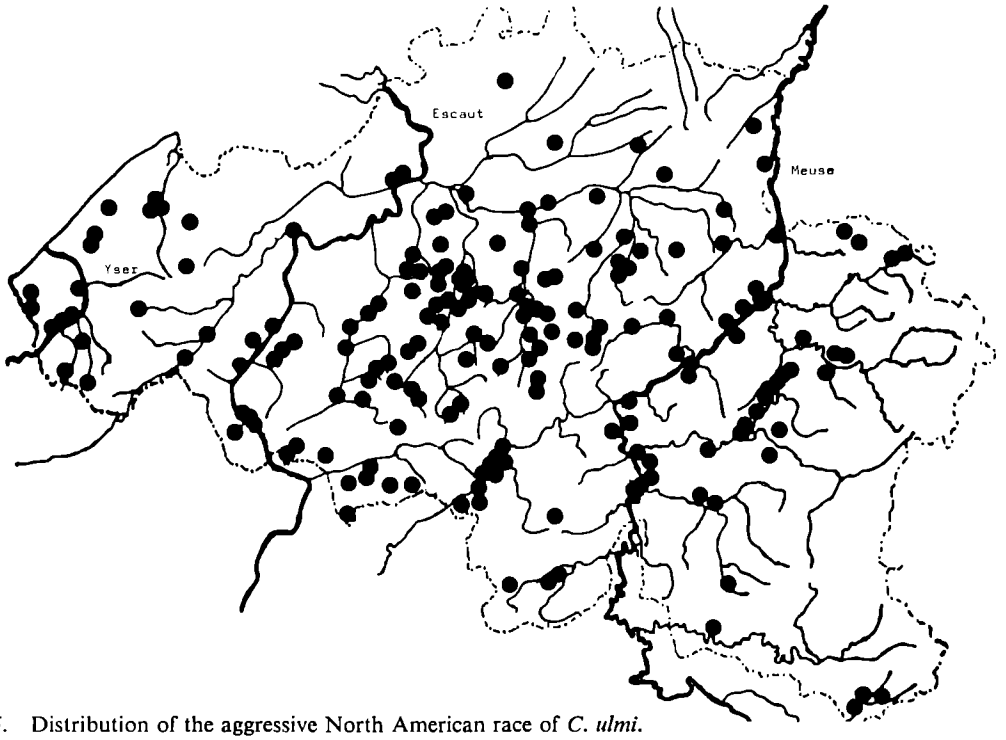
In 1979, we found an isolate of the Eurasian race of the aggressive strain (Meulemans, 1980) in south-east Belgium. This was the first observation of this race in the north-western part of continental Europe. The same race has now also been found in the south of the Netherlands (Brasier, 1981).

Among the 205 aggressive isolates used for crosses in microtiter plates, 161 (78.5 per cent) belonged to the NAN race (140 being of sexual type B, and 21 of sexual type A), and 44 isolates (21.5 per cent), all of sexual type B were of the EAN race. No EAN isolate belonging to type A was discovered in Belgium. The mean growth rate of EAN isolates (4.9 mm/day) differs significantly from that of NAN isolates (5.6 mm/day). Morphologically, the EAN race is distinguishable from the NAN race in that the colonies show an irregular outline, rarely petaloid, with a very frequent appearance of non-sporulating powdery variants. The geographical distribution of the different strains or races of *C. ulmi* in Belgium is represented on maps 4 to 6.

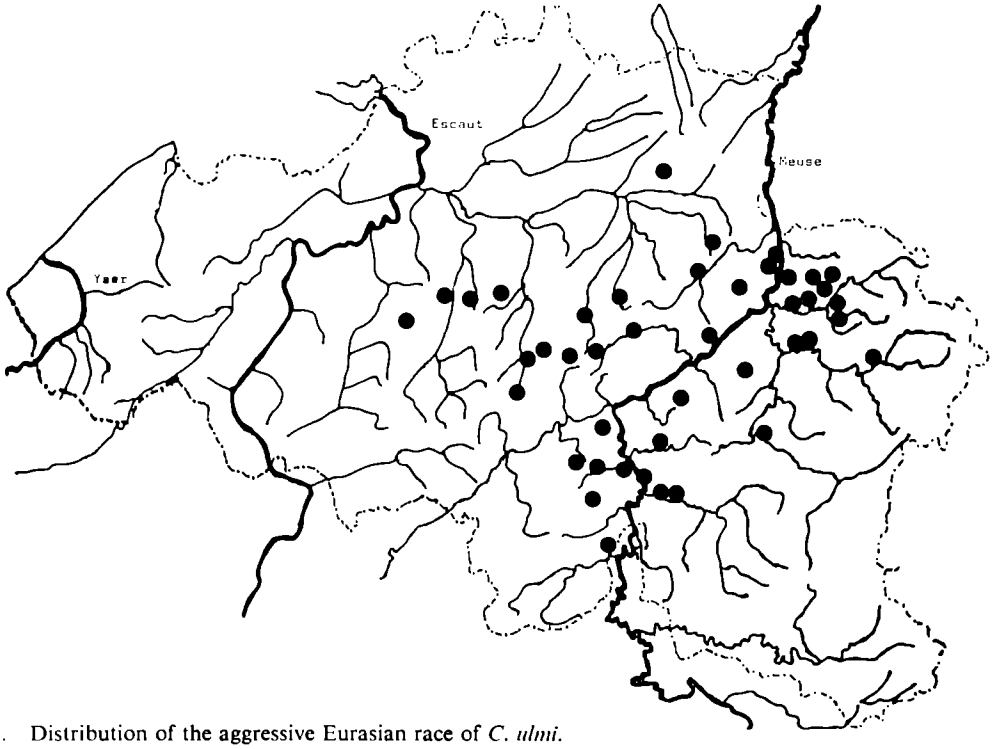
The NAN race of the aggressive strain together with the non-aggressive strain, was distributed throughout the whole country, whereas the EAN race of the aggressive strain was only found in the eastern part of Belgium, in the triangle Brussels–Couvin–Verviers. Moreover the latter race is abundant east of Liège, in the region of Verviers, Stavelot, and Spa. It is possible that the EAN race came from Germany, along the railroad from Köln to Verviers and Liège. Between 1979 and 1981 we



Map 4. Distribution of non-aggressive strain of *C. ulmi*.



Map 5. Distribution of the aggressive North American race of *C. ulmi.*



Map 6. Distribution of the aggressive Eurasian race of *C. ulmi.*

Table 3. Results of inoculation of *U. glabra* and *U. campestris* seedlings with different strains of *C. ulmi*.

Isolate number	Source	Strain	Race	Compatibility type	Percentage defoliation 3 months after inoculation (mean of four replicates)	
					U. glabra	U. campestris
5	Bruxelles	aggressive	N.A.	B	100%	100%
9	Quenast	aggressive	N.A.	B	100%	100%
67	Visé	aggressive	N.A.	B	100%	100%
7	Tournai	aggressive	N.A.	A	100%	100%
11	Drongen	aggressive	N.A.	A	100%	100%
63	Mont-St-Guibert	aggressive	N.A.	A	100%	100%
71	Polleur	aggressive	E.A.	B	80%	40%
397	Goé	aggressive	E.A.	B	90%	50%
409	Morville	aggressive	E.A.	B	100%	90%
12	Temse	non-aggressive	–	n.d.	50%	30%
32	Boiselle	non-aggressive	–	n.d.	100%	40%
72	La Hulpe	non-aggressive	–	n.d.	60%	20%

N.A. = North American race. E.A. = Eurasian race. n.d. = not determined.

have been impressed by the rapid spread of DED along railroads and river valleys, which correspond also to the natural sites where elms are widespread.

In order to study the possible correlations between the characteristics of the isolates (strain, race, mating type) and their aggressiveness, we inoculated 12 isolates (3 NAN type B, 3 NAN type A, 3 EAN type B, and 3 non-aggressive) onto 4-year-old seedlings of *U. glabra* and *U. campestris*. Percentage defoliation observed three months after inoculation is shown in Table 3. All plants inoculated with NAN isolates died, the EAN race was less pathogenic, but was nevertheless more virulent than the non-aggressive strain. The results also showed that *U. glabra* seedlings were more sensitive to DED than those of *U. campestris*.

The finding of double-stranded RNAs in the mycelium of *Endothia parasitica* (Murr.) And. (Dodds, 1980) associated with the phenomenon of hypovirulence, prompted us to search for such ds-RNAs in *C. ulmi*. The analysis by polyacrylamide gel electrophoresis of the RNAs extracted by the technique of Morris and Dodds (1979) permitted us to characterize two peaks of ds-RNA in all three isolates belonging to the NAN race, the EAN race, and the non-aggressive strain respectively. The double stranded structure of these RNAs was ascertained by resistance to RNAs in saline buffer, and sensitivity to the same enzyme in water.

Historical background

When placed in the context of past DED outbreaks in Belgium (Meulemans, 1981) our results raise a number of questions: Has the non-aggressive strain always existed in our elm populations? Was the local dieback of elms observed during the last century due to this strain?

About 50 per cent of elms died in Belgium from 1918 to 1940 while in recent times more than 90 per cent of the elms were killed in less than 10 years. During the first outbreak (1918–1940), young elms about 10 years old, were killed in less than one growing season under the most severe conditions, but older elms died only after two, and often three to four years. Pictures taken during this period showed diseased elms bearing many suckers on the trunk. Furthermore, numerous cases of recovery were observed in Belgium during this period. During the recent outbreak of DED, dieback of elms was very rapid, both for the younger trees and for the valuable older elms. Natural recovery of diseased elms attacked by the NAN race was not observed.

Brasier (1981) reports that the defoliation induced in infected trees was less for the EAN race than for the NAN race. Furthermore, he also observed that recoveries were more frequent for those trees infected by the EAN race than for those attacked by the NAN race. The same tendency was observed in our inoculation tests (Table 3), and we have also noticed the less virulent aspect of the disease in the region where only the EAN race was isolated (Verviers–Spa–Polleur). These data collected during the first and the present outbreaks of DED suggest that the 1918–1940 epidemic might be due to an aggressive strain resembling the EAN race (based on symptoms), while the epidemic of the 1970s resulted mainly from infections by the NAN race.

This succession of outbreaks and decline of DED could be explained by a phenomenon of hypovirul-

ence, which, unlike that observed for *Endothia parasitica* (Dodds, 1980; Grente, 1978), would not be associated with exclusion factors. In fact the frequent appearance of variants and the irregular growth and shape of the colonies might be due to ds RNAs or to satellite viruses as recently shown for *Gaeumannomyces graminis* (Sacc.) Arx et Olivier (Romanos *et al.*, 1981). Although aggressiveness has been attributed to nuclear factors, and seems to be polygenic (Brasier and Gibbs, 1976; Brasier, 1977), the multiplication of some ds-RNA in a given isolate could well modify its pathogenic expression. This aspect will be investigated in further studies of *C. ulmi.*

Control of Dutch elm disease

Felling and destruction of the bark of dead elms, as prescribed by law ("Arrete Royal" of 25th August 1971) has not been carried out either by the private, or by the public owners. The basal numbers of shoots of elms on slopes and hedgerows together with the absence of sanitation felling made it impossible to slow the outbreak of Dutch elm disease. The use of efficient insecticides against bark beetles is forbidden in Belgium. A possible control method lies in the use of pheromones.

The use of fungicides

The only practical measure of control appeared to be the preventive injection of systemic fungicides in the stem to save valuable individual specimen trees. However, these treatments failed occasionally and, as fungicide resistant strains of *C. ulmi* have been found in other countries, we made tests to compare the effectiveness of different fungicides on the growth of the fungus *in vitro.*

The fungistatic activity against *C. ulmi* of a bacterium isolated from elm twigs was also investigated. All experiments were undertaken using two strains of *C. ulmi* (aggressive and non-aggressive) grown on malt medium and incubated in a growth cabinet at 24°C under 16 hours of illumination each day.

Four systemic fungicides were tested: Arbotect and Lignasan, already used for the treatment against DED, and Tilt and Imazalil usually used to control cereal diseases and which proved effective against *C. ulmi* in preliminary tests. The radial growth of the fungal colonies on a medium supplemented with fungicide, and the size of the inhibition zone induced by each fungicide, were used as parameters. *C. ulmi* was inoculated onto malt extract agar containing a given fungicide (final concentration 0.1 to 0.5 ppm). Table 4 shows the results expressed as the main daily radial growth of the colonies. Also filter paper discs were soaked in fungicide and placed in Petri dishes previously inoculated with a spore suspension of *C. ulmi.* After 3 days, the fungus colonized the surface of the medium with the exception of a circular zone surrounding the disc. The results expressed as the mean radius of the inhibition zone are shown in Table 5.

From Tables 4 and 5, we can conclude that Lignasan was more efficient against fungal growth *in vitro* than the other fungicides tested; it is active at lower concentrations and diffuses well in the medium. On the other hand, Tilt does not diffuse well, but is active at rather low concentrations. Furthermore, with these fungicides, and mainly with Tilt, sporulation was also markedly inhibited.

During these experiments, we noticed the development of some *C. ulmi* colonies scattered in the inhibition zone. To test for possible fungicide resistance we compared the radial growth rate of these isolates on a medium containing fungicide, to the rates observed on the same medium for isolates originating from the non-inhibited zone. It can be concluded from the results in Table 6 that, except for Lignasan and Arbotect there was no cross resistance between the different fungicides tested.

Table 4. Mean daily radial growth (in cm) of *C. ulmi* on a fungicide supplemented medium.

Fungicide	Nature of C. ulmi strain	Concentration of fungicide (in ppm)						
		0	0.02	0.05	0.1	0.2	1	5
Lignasan	aggressive	0.43	0.40	0.41	0.30	0.12	0	0
	non-aggressive	0.41	0.36	0.32	0.20	0.07	0	0
Arbotect	aggressive	0.44	0.42	0.41	0.36	0.36	0.06	0
	non-aggressive	0.32	0.33	0.33	0.25	0.24	0.04	0
Imazalil	aggressive	0.43	0.42	0.42	0.41	0.33	0.11	0
	non-aggressive	0.42	0.41	0.40	0.38	0.33	0.07	0
Tilt	aggressive	0.41	0.33	0.29	0.24	0.23	0.06	0
	non-aggressive	0.19	0.16	0.16	0.15	0.11	0.07	0

Table 5. Mean radius of the inhibition zone (in cm) around a fungicide impregnated disc on a medium seeded with *C. ulmi*.

Fungicde	Nature of C. ulmi strain	Concentration of fungicide (in ppm)					
		10	50	100	500	1,000	2,500
Lignasan	aggressive	0	1.05	1.20	1.65	2.05	2.60
	non-aggressive	0	0.85	1.30	1.70	2.35	2.85
Arbotect	aggressive	0	0.55	0.72	1.35	1.75	2.15
	non-aggressive	0	0	0.80	1.50	1.75	2.35
Imazalil	aggressive	0	0	0.75	1.25	1.46	2.13
	non-aggressive	0	0	1.03	1.60	1.81	2.73
Tilt	aggressive	0	0	1.15	1.30	1.46	1.65
	non-aggressive	0	0	0.66	1.50	1.51	1.85

Failures observed with some Lignasan treatments could be due to the appearance of resistant *C. ulmi* variants. This resistance was maintained after serial transfers on a fungicide-free medium, and such variants would probably also be stable in nature.

Antagonistic bacteria

Our isolations of *C. ulmi* were always made from pieces of elm twigs. Many times, bacteria originating from these twigs developed on the malt agar medium, preventing the spread of the fungus over the whole surface. As the fungal growth was inhibited at a distance from bacterial colonies, the existence of a fungistatic substance emitted by the bacteria was suspected (Figure 2).

The five more active strains of antagonistic bacteria (acting at more than 5 mm from the margin of their colony) were selected; they were all identified as *Bacillus subtilis* isolates. We tested the antifungal activity of filtrates from bacterial cultures by incorporating them in solid medium. The bacteria grew well, and produced fungistatic substances on malt extract agar or on agar medium containing elm wood, but grew poorly and did not produce fungistatic substances in liquid nutrient broth or on nutrient agar or Bacto agar. Figure 3 shows that a bacterial filtrate was inhibitory when assayed after 6 or 7 days of culture, but not after 3 days.

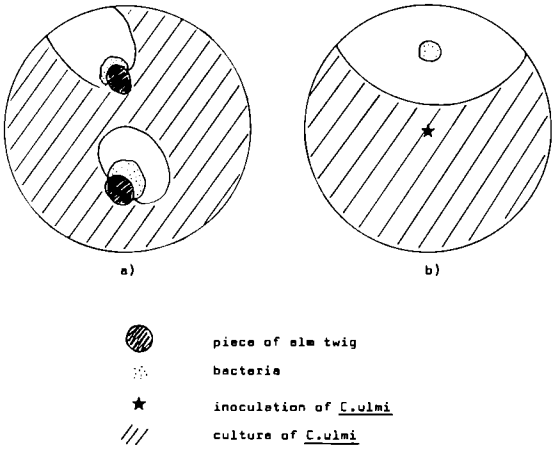
We also noticed that *C. ulmi* surviving a sub-lethal dose of toxic filtrate was much less sensitive

Table 6. Estimation of the growth of different resistant strains of *C. ulmi*.

Strain grown in presence of	Control	Concentration of fungicide (in ppm)							
		Lignasan			Arbotect			Imazalil	Tilt
		100	500	1,000	100	500	1,000	all concentrations	
Control (aggressive strain)	+++	—	—	—	—	—	—	—	—
Control (non-aggressive strain)	++	—	—	—	—	—	—	—	—
Lignasan 2,500 ppm (aggressive strain)	+++	+	+	+	++	—	—	—	—
Lignasan 100 ppm (non-aggressive strain)	++	—	—	—	—	—	—	—	—
Arbotect 2,500 ppm (aggressive strain)	+++	+	+	+	++	—	—	—	—
Arbotect 500 ppm (aggressive strain)	+++	+	+	+	++	—	—	—	—
Arbotect 1,000 ppm (non-aggressive strain)	++	—	—	—	—	—	—	—	—
Arbotect 100 ppm (non-aggressive strain)	++	—	—	—	—	—	—	—	—

+++ normal growth. ++ slow growth. + poor growth. — no growth.

to a subsequent treatment with the filtrate, even if the strain had been grown in the meantime on non-toxic malt extract agar (Figure 4). It remains to be shown whether the bacterial filtrate is also active *in vivo* and not phytotoxic to the elm.



a) bacteria and *C. ulmi* isolated together from pieces of elm twigs.

b) development of the growth of *C. ulmi* inoculated in the presence of the bacteria.

Figure 2. Description of the inhibition produced by the bacteria.

Development of a simple injection device for the treatment of elms

In order to provide treatment by a simple technique which would permit its use by each owner of elm trees, we have developed a simple gravity feed injection mechanism. Injectors linked together by rubber tubing are placed at the base of the trunk: these injectors are connected to the bottom of a bottle containing the fungitoxic solution which is attached higher up on the trunk. This technique has the advantage of utilising cheap and simple material. However, absorption by the tree is slow and depends on the evapotranspiration rate of the tree, on the climate and on the formulation of the product. For example, in this latter respect, results for Tilt were negative because of the formation of a precipitate in the tubing and at the bottom of the bottle.

To conclude, much remains to be done in order to obtain an efficient and not too expensive method for the control and for the prevention of DED. Furthermore, as our only product approved against DED, Lignasan, has been withdrawn from the Belgian market, it is now imperative that other remedies are found to protect our few remaining elms.

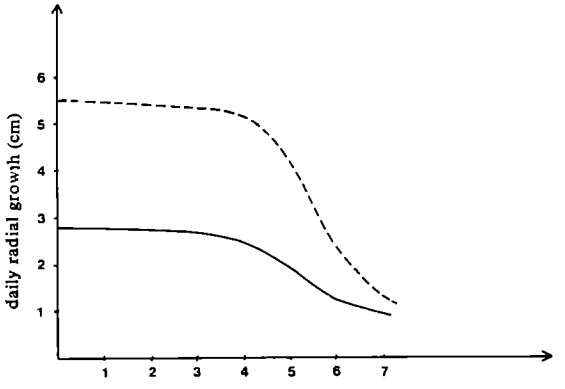


Figure 3. Mean daily growth of *C. ulmi*, cultivated on malt extract agar containing bacterial filtrate, as a function of the age of the bacterial culture before filtration.

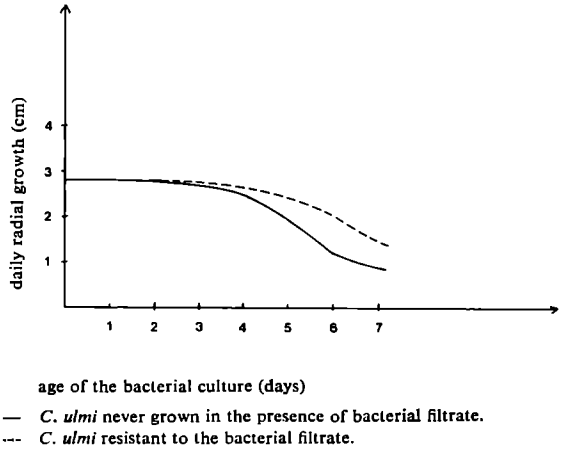


Figure 4. Mean daily radial growth of resistant *C. ulmi* and non-resistant *C. ulmi* cultivated on malt extract agar containing bacterial filtrate.

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Maps 1 to 6 and Tables 1 to 3 are presented in Meulemans *et al.* (1981).

PAPER 18

The future of Dutch elm disease in Europe

C. M. BRASIER

Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH, U.K.

Introduction

Forest pathologists and entomologists are usually well able to measure the rates of spread of disease outbreaks and to conduct post-mortem investigations into their causes, but are rarely able to predict their occurrence in advance or to intervene positively. All too often, therefore, our role is limited to that of being mere observers or to tinkering with events, the principal reason being that our biological knowledge of the pathogen or pest and of the biological processes underlying epidemics is too weak.

In the present epidemic of Dutch elm disease the underlying cause can be identified with hindsight: there has been a change in the behaviour of the pathogen, *Ceratocystis ulmi*. By the same token the future behaviour of the pathogen must hold the main key to the future of the elm, of the vector beetles and of the effectiveness of our control measures, in particular breeding for disease resistance. The question I should like to address in this paper is whether our still limited knowledge of the pathogen's biology enables us to predict the pathogen's future and hence the future of Dutch elm disease in Europe.

Causes of the present epidemics

The sub-groups of Ceratocystis ulmi

During the past decade we have been forced to learn rather quickly that *Ceratocystis ulmi* exists not as a continuum of variation within one population, but as three reproductively isolated sub-populations or sub-groups (Figure 1): the highly pathogenic aggressive strain, which itself divides into two separate races, the Eurasian (EAN) and North American (NAN) races; and the more weakly pathogenic non-aggressive strain (Gibbs and Brasier, 1973; Brasier, 1979). The aggressive and non-aggressive strains differ in most important cultural and physiological characteristics, and should probably be considered sub-species (Brasier, 1982a). The two races of the aggressive strain also differ in many characteristics, but their similarities broadly outweigh their differences. The properties of the sub-groups have been summarised elsewhere (Brasier, 1982b).

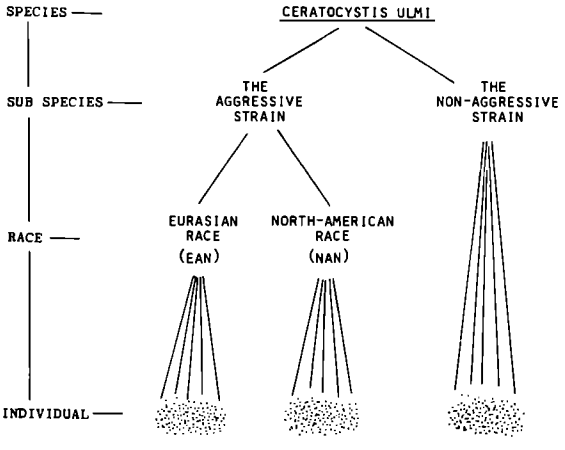

Figure 1. The division of *C. ulmi* into three reproductively isolated sub-groups or sub-populations. Each sub-group has its own characteristics and range of variation.

The changes in the Ceratocystis ulmi population

The current outbreaks of Dutch elm disease across Europe are the result of a major change in the structure of the *C. ulmi* population. The areas once occupied only by the non-aggressive strain (believed to have been responsible for the first epidemic of Dutch elm disease in the 1920s and 1930s) are under invasion by one or other race of the aggressive strain. This phenomenon was first recognised along with the identification of the aggressive and non-aggressive strains in England in the early 1970s (Gibbs and Brasier, 1973) but it is also occurring in most other European countries, in eastern North America and in south-west Asia.

More specifically, the current disease outbreaks in Europe are thought to be due to two separate events:

1. The importation of the NAN race of the aggressive strain from North America into Britain during the mid-1960s (Brasier and Gibbs, 1973), and its subsequent spread into neighbouring countries of north-west Europe (Brasier, 1979).
2. The westward migration of the EAN race of the aggressive strain from central and southern Europe or from further east (Brasier, 1979).

The present situation in Europe is developing rapidly as the EAN race spreads westwards and the NAN eastwards, and in some ways parallels the situation which occurred during the first epidemic in the 1920s when the disease spread outwards from a centre close to northern France (although the present outbreaks are undoubtedly of far greater severity). To obtain an accurate picture of the present situation in Europe, detailed ground surveys of the sub-groups of *C. ulmi* have been carried out in a number of centres from Poland to eastern Turkey, linking with surveys carried out previously in Iran (Brasier and Afsharpour, 1979), the U.S.S.R. (C. M. Brasier, unpublished) and North America (Gibbs, Houston and Smalley, 1979). The results of the surveys will be published elsewhere. The present known positions of the EAN and NAN races of the aggressive strain in Europe are summarised in Figure 2.

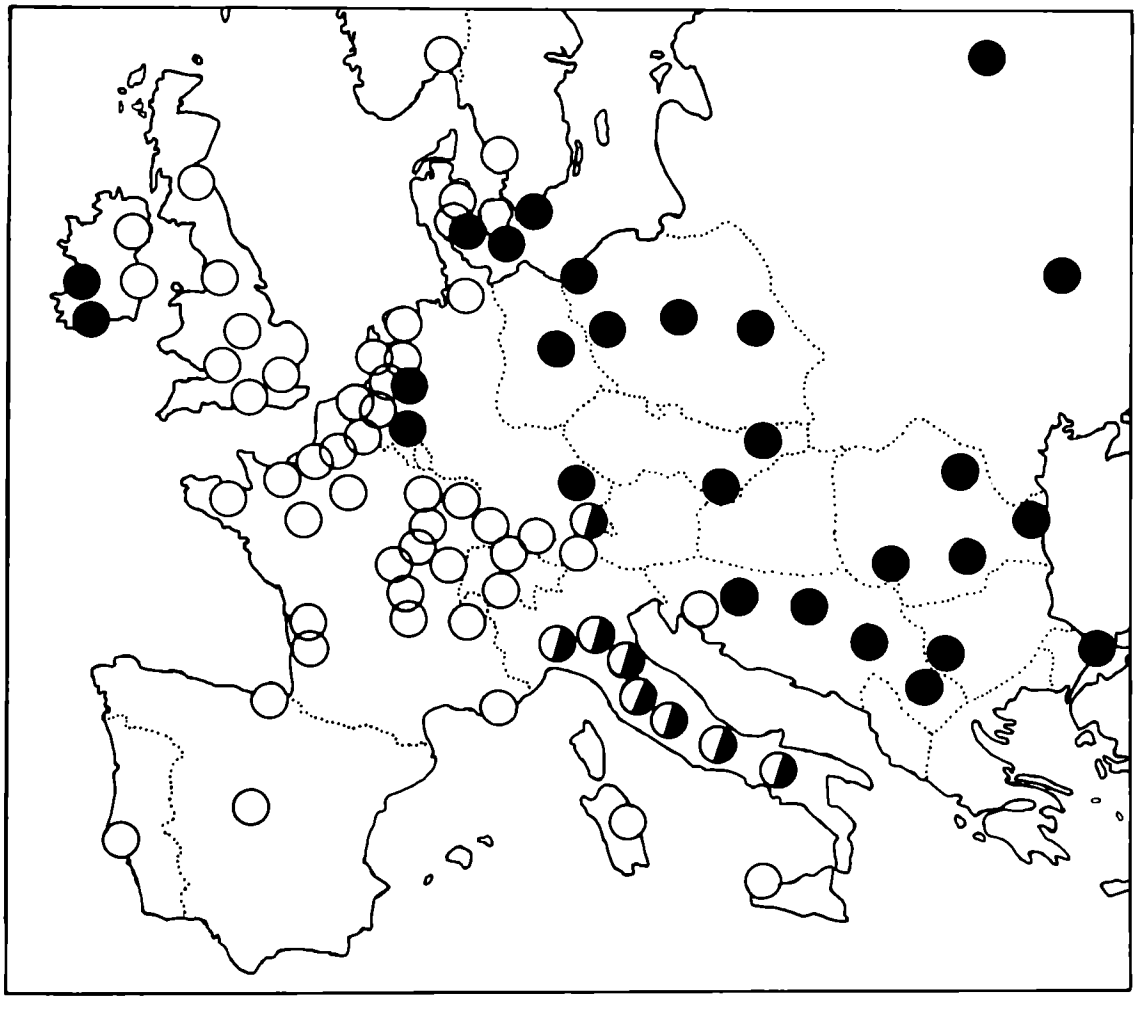

Figure 2. Summary of the known positions of the EAN and NAN races of the aggressive strain of *C. ulmi* in Europe in 1981. O, NAN race; ●, EAN race. Based on >1000 samples collected by the author. The distribution of the non-aggressive strain is not shown.

The Future

Rather unusually, Europe is experiencing the simultaneous development of two separate epidemic events, one coming from the east and one from the west. The events are now overlapping to the extent that the two races of the aggressive strain can now be found side by side in a number of countries (Figure 2). In some parts of Europe such as Britain and Romania, extremely heavy losses have already occurred. Thus in Britain some 20 million elms have died during the 1970s alone. In other countries such as Spain and Sweden, the losses are only just beginning. The whole episode may well last some forty or fifty years.

In these circumstances, what does the future hold for the next generation of European elms, the healthy young seedlings and root suckers which are now developing, sometimes in great numbers, in areas where the large elms have recently died from the disease? Will the disease return and destroy these elms when they are large enough to support a population of breeding beetles? The answer to this question depends in turn upon the future behaviour of the aggressive strain: will it decline or die out in the face of the collapsing host and beetle population, or will it survive? At present there appear to be three ways in which the decline of the aggressive strain might come about:

1. Through a reduction in the pathogenicity of the aggressive strain itself via internal genetic mechanisms.
2. Through hybridization with the non-aggressive strain.
3. Through its replacement by the non-aggressive strain.

These possibilities will now be considered.

Reduction in pathogenicity of the aggressive strain through internal genetic mechanisms

Present knowledge is insufficient to predict the likelihood of attenuation in pathogenicity of the aggressive strain itself. However, from the limited information available it is possible to suggest how such attenuation might occur and to consider some of its consequences.

The aggressive strain should not be considered as a single unit for this purpose since there are a number of potentially important differences between the EAN and NAN races, including differences in pathogenicity. There is a greater range of pathogenic ability within the EAN race than within the NAN, and EAN isolates are less likely to cause disease recurrence in a second season (Brasier, 1982a). In the post-epidemic situation, therefore, the EAN may have a greater capacity to respond to the selection imposed by the reduced host and beetle population, e.g. through the survival of its less pathogenic elements. It may also be better equipped to survive in direct competition with the NAN race where the two occur together (as for example in Denmark, Ireland and Italy, see Figure 2). Mutation, and hybridization within and between the EAN and NAN races are other factors likely to contribute to attenuation in the post-epidemic situation, assuming that more weakly pathogenic mutants or recombinants would be selected for. Although a sterility barrier is operated by the EAN race against the NAN, the barrier is only partial (Brasier, 1979) and as such is more likely to reduce than to prevent EAN x NAN hybridization in nature.

Although pathogenicity in *C. ulmi* is thought to be largely under the control of nuclear genes (Brasier, 1977, 1982a) attenuation might also come about through the increased influence of those cytoplasmic factors tending to moderate the expression of pathogenicity such as the *'up-mut'* and 'd' factors (Brasier, 1982b and unpublished), mitochondrial mutants, dsRNA components (Pusey and Wilson, 1982) and virus-like particles, c.f. cytoplasmically transmitted hypovirulence in the Chestnut blight fungus, *Endothia parasitica* (Anagnostakis, 1982). In response to strong directional selection cytoplasmic factors such as viruses could conceivably bring about a fairly rapid reduction in pathogenicity, but since they may also tend to reduce fitness in other survival characters such as reproductive vigour, they are likely to be reinforced or superseded by nuclear gene modifications to the pathogenicity system via mutation and recombination.

Ultimately, attenuation might lead to the emergence of a further more moderately pathogenic form of the fungus in better balance with the host population. It would probably be better adapted to the post-epidemic situation in a number of other ways, and would hence be unlike the aggressive or non-aggressive strains as we know them. In this case it would be a distinct sub-group of *C. ulmi* in its own right. It might first appear as a successful variant in a 'hybrid zone' where the EAN and NAN races overlapped.

Whatever the possibilities, both the probability of and a likely time-scale for attenuation are unknown. Further research is therefore needed on the nature and intensity of the selection imposed on the aggressive strain by a declining host and beetle population; on the genetic basis of the pathogenicity differences between the EAN and NAN races; and on the potential for hybridization between them and its likely outcome. Information

is also required on the characteristics and influence of cytoplasmic components of *C. ulmi* (such as viruses) on pathogenicity and on their potential for transmission from one individual to another, for example across hyphal anastomoses.

Hybridization of the aggressive and non-aggressive strains

There is no doubt that in epidemic outbreak areas sexually compatible isolates of the aggressive and non-aggressive strains can be obtained from the same piece of diseased elm bark (Brasier and Gibbs, 1976). Therefore there is certainly the physical possibility of hybridization occurring, especially during the earlier stages of an epidemic (see later). However, we now also have enough biological evidence to predict that such hybridization is unlikely to be an important factor in the decline of the aggressive strain. This evidence is of four main types.

Firstly, when crosses are made between the aggressive and non-aggressive strains in the laboratory, the resulting progeny are quite unlike their parent types, being extremely unusual in cultural characteristics and, most importantly, generally rather weak pathogens. Although they might therefore be expected to contribute to a decline in pathogenicity of the *C. ulmi* population their various characteristics also indicate that they are likely to be generally unfit and are unlikely to survive long as pathogens in nature (Brasier and Gibbs, 1976; Brasier, 1977, 1982a). Secondly, although such crosses can be forced in the laboratory, other laboratory experiments have shown that a reproductive barrier exists which largely prevents the aggressive strain from being fertilised by the non-aggressive strain in aggressive ♀ x non-aggressive ♂ pairings (Brasier, 1977). Thirdly, experiments using mites as the fertilising agents to reproduce conditions close to those occurring in nature indicate that in nature the barrier to hybridization would be virtually total (Brasier, 1978).

The fourth line of evidence comes directly from nature. Although many thousands of fresh wild isolates of *C. ulmi* have been examined by the author from North America to south west Asia from localities where both the aggressive and non-aggressive strains occur side by side, only a very few isolates have been seen which cannot be confidently assigned to either the non-aggressive or one or other race of the aggressive strain. The few isolates have usually conformed to mutant types of the normal aggressive and non-aggressive strain wild types, and have not had the characteristics of hybrids. Thus on present evidence it appears that hybridization between the aggressive and non-aggressive strains is likely to be a rare event, and even if it does occur the progeny are unlikly to survive and there is no evidence of them doing so. It therefore seems reasonable to suggest that such hybridization will not be involved in any future decline in pathogenicity of the aggressive strain or of the the *C. ulmi* population as a whole.

Replacement of the aggressive by the non-aggressive strain

I should like to look in more detail at the possibility that the aggressive strain may again be replaced by the non-aggressive strain as the elm population declines, and to begin by considering the relationship between the two strains from the moment that the aggressive strain arrives in territory previously occupied only by the non-aggressive strain. The following is put forward as the possible course of events during the early stages of an epidemic (for further details see Figure 3).

- When the aggressive strain is first introduced into an area (Figure 3A) only the non-aggressive strain is being carried by the local beetle population. The first tree(s) killed by the aggressive strain will therefore be used as breeding material mainly by local beetles carrying the non-aggressive strain.
- A large number of beetles will therefore leave this tree carrying the non-aggressive strain, resulting in an increase in the number of infections caused by the non-aggressive strain, and also the spread of the aggressive strain. In this way, we may expect at the beginning of an epidemic an initial build-up in the frequency of the non-aggressive strain to well above its previous (unspecified) level (Figure 3B). Thus the non-aggressive strain may initially benefit from the arrival of the aggressive strain.
- As the aggressive strain gradually increases in frequency through killing more trees and providing more beetle breeding material the numbers of infections initiated by the non-aggressive strain are overtaken by those initiated by the aggressive strain (Figure 3C).

Evidence for a benefit to the non-aggressive strain comes from sample data which show a remarkably high proportion of non-aggressive strain infections in the early to mid-term stages of recent disease outbreaks. Thus, in two of the three main disease outbreak areas in Britain during 1971, the non-aggressive strain accounted for over 22 per cent of infections (Gibbs and Brasier, 1973 and see Table 1) an infection rate probably well above that which would have occurred if the non-aggressive strain was present on its own.

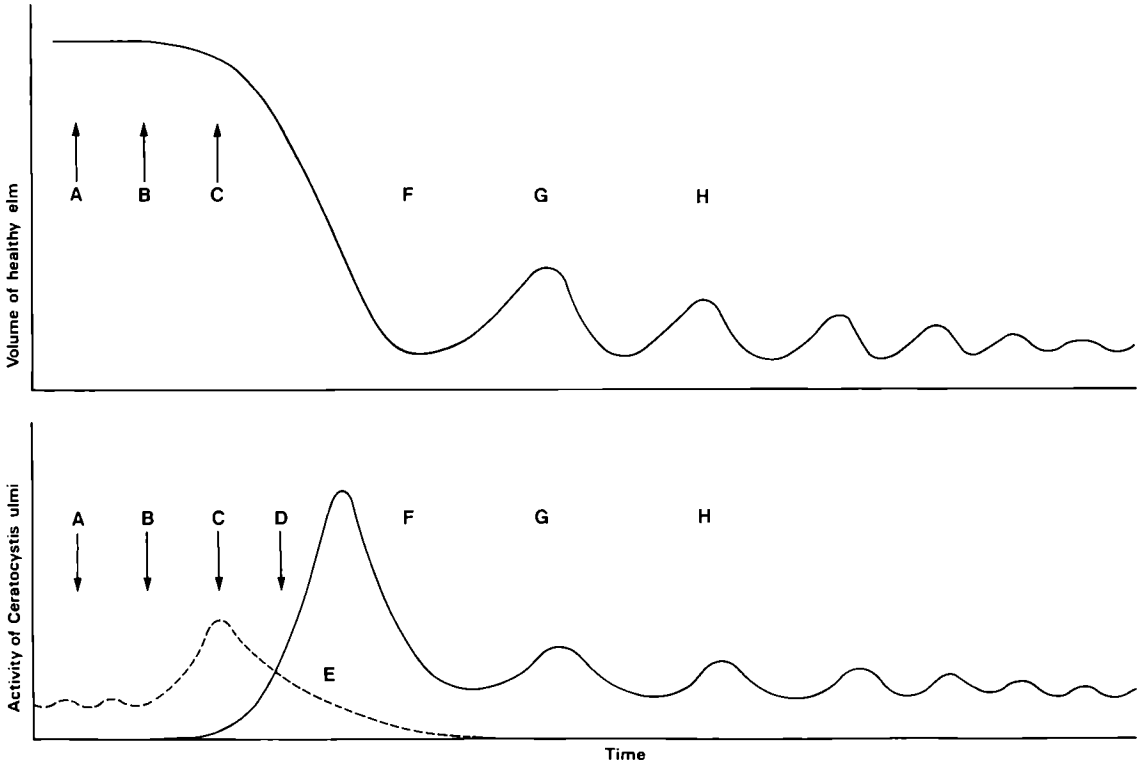

Figure 3. Suggested course of events in the recent Dutch elm disease outbreaks in Europe and their projected outcome. Upper graph, changes in the European elm population. Bottom graph, changes in the *C. ulmi* population: ----, non-aggressive strain; ——, aggressive strain.

A. Prior to the arrival of the aggressive strain, the elm population is subject to periodic flare-ups of the non-aggressive strain.

B. The aggressive strain arrives in a locality. The first tree killed by the aggressive strain is used as breeding material by local beetles already carrying the non-aggressive strain. A large number of beetles leave this tree also carrying the non-aggressive strain, and some the aggressive strain.

C. This process results in an increase in frequency of infections caused by the non-aggressive strain to well above its previous level, and a gradual increase in the number of trees killed by the aggressive strain.

D. Through killing more trees and providing a greater volume of beetle breeding material, infections initiated by the aggressive strain begin to increase, rapidly overtaking those initiated by the non-aggressive strain.

E. The non-aggressive strain goes into decline.

F. Most of the accessible large elms are killed, resulting in a collapse of the beetle population and that of the aggressive strain. The non-aggressive strain is by now virtually eliminated in the main epidemic areas. It survives in a few isolated pockets of elm untouched by the aggressive strain.

G. Elm seedlings and root suckers regenerate in large numbers. When large enough to support beetle breeding, they are attacked by the aggressive strain. Due to the marked reduction in the size of available breeding material, the principal beetle vector *Scolytus scolytus* goes into a decline, and the smaller beetles such as *S. multistriatus*, *S. kershi* and *S. ensifer* become of major importance in disease transmission.

H. The cycle is repeated. Field elms are largely reduced to a scrub or understorey population. Most surviving mature trees are escapes in woodlands, on islands and in upland valleys.

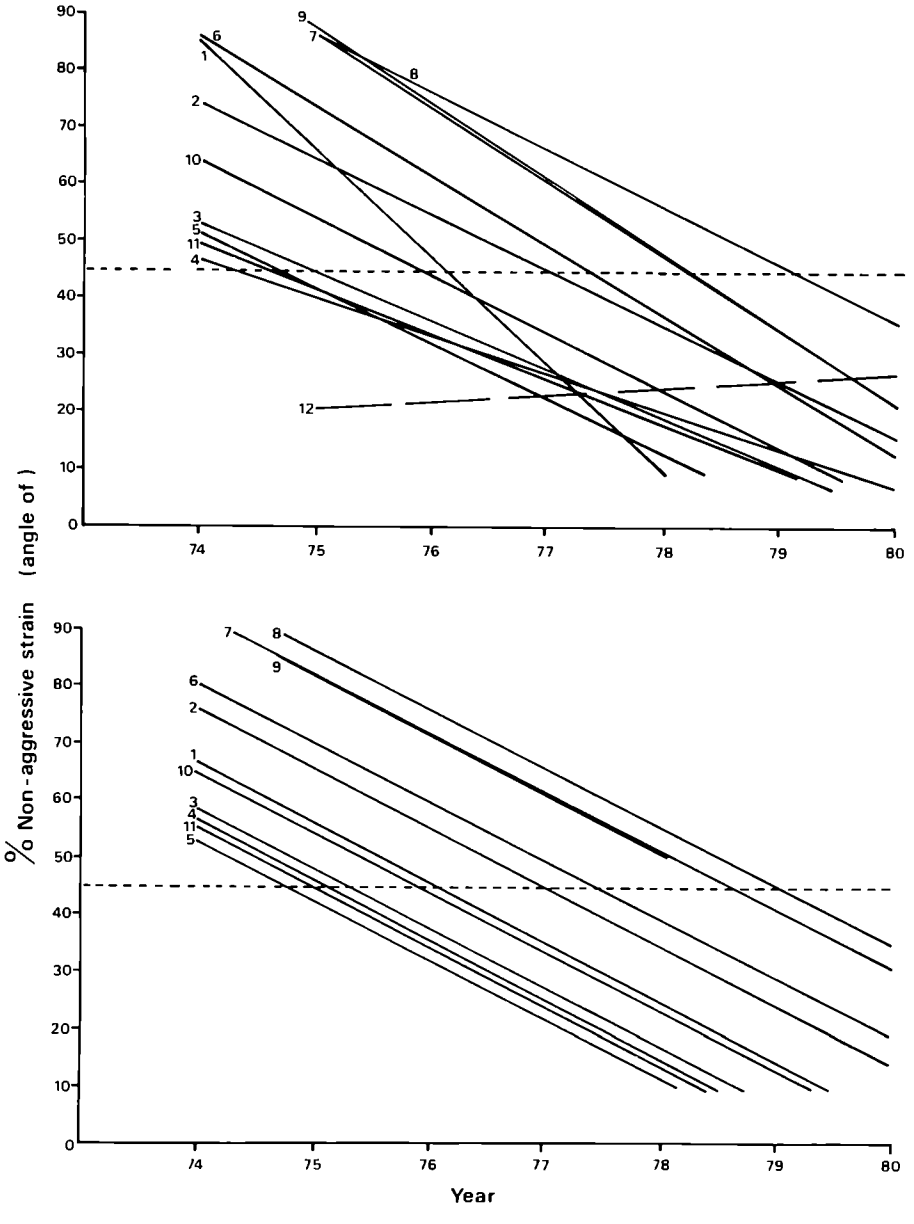

Figure 4a. Regression slope showing the decline in the non-aggressive strain in the twelve provinces of Holland between 1974–1980. The dashed line indicates the 50% position (transformed). Based on a total of 1,300 samples (Gremmen, Heybroek and de Kam, 1976; C. M. Brasier and H. M. Heybroek, unpublished data). The slopes show significant interactions (P = <0.05). The provinces are as follows: 1, Groningen; 2, Friesland; 3, Drenthe; 4, Overijssel; 5, Gelderland; 6, Utrecht; 7, Noord-Holland; 8, Zuid-Holland; 9, Zeeland; 10, Noord Brabant; 11, Limburg; 12, Flevoland.

Figure 4b. The same data with Province number 12 (Flevoland) omitted. There are now no significant interactions between the regression slopes, which are plotted here according to the mean slope. Note the rapid decline in the proportion of the non-aggressive strain in all eleven provinces. Note also that the four most eastern provinces (3, 4, 5, 11) reached the 50% level several years ahead of the three most western provinces (7, 8, 9) indicating that the aggressive strain arrived earlier in the east, probably via Germany.

Table 1. Changes in the frequency of the aggressive and non-aggressive strain of *Ceratocystis ulmi* at four localities in Britain between 1971 and 1978.

Locality	Year	Number of samples yielding		% Non-aggressive
		Non-aggressive strain	Aggressive strain (NAN)	
Ilchester, Somerset	1971	5	17	22.7
	1972	3	33	8.3
	1974	1	48	2.0
*Tewkesbury, Gloucestershire	1971	8	28	22
	1972	6	41	12.8
	1972a	4	22	15.4
	1974	1	38	2.6
	1974b	1	48	2.0
	1978b	0	84	0.0
*Chichester, Sussex	1971	9	30	23.1
	1974c	0	18	0.0
	1975	3	50	5.7
	1978c	0	59	0.0
*Orsett, Essex	1971	1	35	2.9
	1972d	1	46	2.2
	1975e	1	29	3.4
	1978d	0	89	0.0

* The three main outbreak areas in Britain identified by Gibbs and Brasier (1973).
a, sample of Berkeley Vale, Gloucestershire; b, sample of whole of Gloucestershire; c, sample of whole of Chichester—Southampton area; d, sample of whole of south Essex; e, sample of Basildon area.

Valuable data is available showing the change in the balance of the aggressive and non-aggressive strains as an outbreak has progressed. The most comprehensive data comes from Holland, where samples have been taken in the twelve individual provinces over the period 1974–1980. Figure 4 shows the plotted data for all twelve provinces. In a regression analysis the slopes show significant interactions. However, there are no interactions if the data for Flevoland (Figure 4a, number 12) are omitted. This allows the remaining eleven provinces to be plotted on the basis of the overall regression slope, as shown in Figure 4b. The results show clearly that there is a steady and dramatic decline in the frequency of the non-aggressive strain in the eleven provinces as the epidemic progresses.

In Britain changes in the frequency of the aggressive and non-aggressive strains have been monitored since 1971 at four sites, including the three original main disease outbreak areas. These data (Table 1) show that by 1971 the non-aggressive strain in Britain had already advanced to a level not reached in many Dutch provinces until 1980. The decline continued until 1978, by which time the non-aggressive strain was less than one per cent of the *C. ulmi* population at all four sites. Regression slopes for the British sites in comparison with a representative site from Holland are shown in Figure 5.

These data indicate that we can expect the virtual disappearance of the non-aggressive strain at some stage during the course of the epidemic (Figure 3D). They do not, however, answer the question of whether as the elm population collapses it will die out completely, or whether it could still return to replace the aggressive strain.

The situation in Romania

Evidence of what may happen to the non-aggressive strain in future may be found in Romania. Although many of Romania's elms survived the first epidemic of the disease in the 1930s, a second more severe epidemic of the disease occurred in Romania as early as the 1950s (Petrescu *et al.*, 1963) beginning in the Moldavian plateau region around Iasi. In the second outbreak most of the elms, including the many elms that occurred in mixed stands with oak in the forests of Moldavia, were killed by the disease.

To investigate this situation, the author conducted a sample survey of *C. ulmi* in Romania in 1980. The results showed that the EAN race of the aggressive strain was present, consistent with the postulated westward spread of the EAN race across

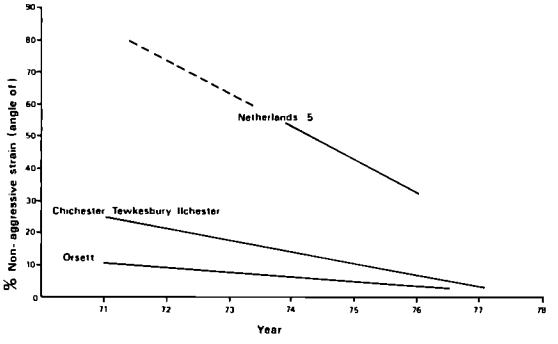

Figure 5. Regression slopes for changes in the proportion of the non-aggressive strain at sites in Britain between 1971–1978 (based on the data in Table 1) and a regression slope for a representative site in Holland. The non-aggressive strain, which had already reached a low frequency at the British sites by 1981, continued to decline to a scarcely detectable level by 1978. The data also indicate (i), that the outbreak in Orsett may have been several years ahead of that in Chichester and Tewkesbury and (ii), that if by extrapolation from these data and those in Figure 4b the aggressive strain arrived in the Orsett area some 10–15 years prior to 1971, then the beginning of the British epidemic may have been as early as 1955–1960 (*cf.* Brasier and Gibbs, 1973).

central and eastern Europe in recent years (Brasier, 1979). It is therefore concluded that the second wave of the disease in Romania in the 1950s was due to the arrival of this form of the fungus, and that the original outbreak was caused by the non-aggressive strain.

On this assumption, the present situation in Romania represents a situation 30 years after the arrival of the aggressive strain, and some 10–15 years ahead of the present situation in north-west Europe, and can therefore be taken as a pointer to the future. On this basis, the following important observations were made during the 1980 survey:

1. In the Moldavian plateau region of Romania the elms (mostly *U. carpinifolia*) are largely reduced to bushes and saplings at the margins of forests and in clearings.
2. The disease is still remarkably heavy, even among relatively small saplings.
3. One hundred and twenty samples of the fungus were collected in this region. None of them yielded the non-aggressive strain, the EAN race of the aggressive strain being obtained from all 120 samples.

Conclusions

A number of conclusions may be drawn from the above information regarding the possible future of the elm, of Dutch elm disease and of disease control measures in Europe. They are offered on the basis that some form of prognosis is better than no prognosis at all, and on the understanding that with our knowledge being inadequate the absence of a vital piece of information, whether due to paucity of research or the ignorance of the author, could at any time render them invalid. Specific conclusions are as follows:

1. *That* a decline in pathogenicity of the *C. ulmi* population through hybridization of the aggressive and non-aggressive strains or through replacement of the aggressive by the non-aggressive strain can be discounted.
2. *That* the non-aggressive strain may be heading for virtual extinction throughout many of our disease outbreak areas (Figure 3D, F).
3. *That* the future of our European field elms is bleak. They may be largely reduced to an understorey or scrub population (with occasional escapes especially in mountain valley areas) under recurrent attack by one or other race of the aggressive strain (Figure 3G).
4. *That* the smaller scolytid beetles may become the major vectors of the disease (Figure 3G).
5. *That* any reduction in the pathogenicity of the *C. ulmi* population to meet selection pressure imposed by a reduced host and vector population is most likely to occur through the attentuation of pathogenicity within the aggressive strain itself.

If the above conclusions are broadly correct, then the future of the elm in Europe and elsewhere may parallel the fate of the American chestnut in the wake of Chestnut blight. Once occupying a quarter of America's eastern hardwood forests, the American chestnut has been devastated as a result of the introduction of Chestnut blight from Asia at the turn of the century. It now survives mainly as coppice shoots under continual attack from the disease. With Dutch elm disease, however, we are witnessing not a regional continental event involving a single species, but a massive pandemic across three continents from California through Europe to at least as far as Iran, involving the destruction of many species of elm in many habitats: a colossal natural disaster, perhaps without equivalent in recorded history, which will impoverish a diverse array of human cultures. In the circumstances, it is a sad commentary on our attitude to trees and tree diseases that the steady westward march of the EAN epidemic across Europe over three or more decades has gone virtually unnoticed until now.

It appears that the host pathogen relationship in Europe may remain seriously out of balance unless the aggressive strain in some way attentuates fairly rapidly. Clearly this does not augur well for the control of the disease in the near future. Where we have specific requirements for elm, for example for shade trees in the towns of Italy or for shelter belts in the polders of Holland, the production of disease resistant elms may be our greatest long-term hope of redressing this balance. This does not, however, answer the problem of the loss of our field elms for which we must also take responsibility; it is in the countryside that the real battle of Dutch elm disease control, that for a natural balance between host and pathogen, will always be fought. Serious consideration should perhaps be given to ways of raising the base line of disease resistance in our native European field elms, for example by encouraging exotic disease resistant species with suitable properties (such as forms of *U. japonica*) to interbreed with the wild populations.

In order to breed for resistance with due confidence we must know as precisely as possible what it is we are breeding for resistance to, i.e. the genetic basis of pathogenicity and the full range of pathogenic variation in the fungus. If we are to do this, one vital piece of biological information is missing. We remain in ignorance of the geographical centre of origin – or source – of Dutch elm disease, thought to be in eastern Asia (Heybroek, 1976). The centre of origin may well be the centre of fungal diversity, and hence the source of new strains and races of the pathogen which could attack our European elms, including our disease resistant ones, in future. Thus, while with hindsight we may now talk with some semblance of authority on the causes of our present epidemics in Europe, we may in reality only be mere observers standing on the perimeter of a much larger event centred somewhere in the east.

In this case we are still only speculating on the origin of our epidemic. If we can identify the real source of our troubles then we might better understand the balance of host and pathogen and, armed with better knowledge, begin to shift our emphasis from observation to confident scientific prediction and intervention. To identify the geographical source of *C. ulmi* is both the foundation stone and a fundamental objective of the present EEC project.

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PAPER 19

Dutch elm disease research in Italy

L. MITTEMPERGHER

Centro di Studio per la Patologia delle Specie Legnose Montane del Consiglio Nazionale delle Ricerche, Firenze, Italy

Introduction

The work of breeding elms resistant to Dutch elm disease (DED) undertaken in Florence, Italy, during the past few years has been based upon two considerations: (1) differences in climate under which tests are conducted by American, Dutch and Italian breeders may be reflected in the phenotypes selected and (2) a requirement to introduce resistance into the extant gene pool from the largest number of diverse sources in order to avoid a possible lowering of resistance in the future.

With regard to the first point I would like to stress the good adaptability in central Italy of the southern provenances of *Ulmus japonica* and *U. pumila* which encounter some problems in the Netherlands. Some other species and provenances are expected to grow successfully in Italy as well as in areas of milder climate in Europe.

As for the second point, in October 1981 I presented a paper at the NATO Advanced Study Institute meeting on "Durable resistance in crops" held in Martina Franca, Italy. I noted the existence of elements of verticality in the horizontal pathosystem elm–*Ceratocystis ulmi* because a significant, even if weak, interaction among clones of *U. pumila* and isolates of *C. ulmi* was found (Mittempergher and Raddi, 1981). This statement can perhaps be strengthened by the fact that fungal pathogenicity and host resistance are inherited not simply quantitatively, but show the presence of major and minor genes. In fact Brasier (1980) found qualitative as well as quantitative differences in pathogenicity by crossing the aggressive and non-aggressive strains, which he explained in terms of an operon-type genetic system. Townsend (1979) noticed the existence of special combining ability in several cross-combinations among various species of elm, confirmed by the lack of precise transmission of clonal resistance to seedling progeny. If this analysis is correct, the best defence against a possible fall in resistance in the future is to introduce resistance from many diverse genetic sources.

Some other considerations have been taken into account in designing and carrying out my elm breeding programme: (1) to save if possible the germplasm of *U. carpinifolia* which is a genetically varied, well adapted and appreciated species; (2) to make use of the breeding work already undertaken by including good hybrids in more complex combinations; (3) to select also for resistance to *Galerucella luteola* Müll., a leaf-eating beetle which occasionally is very destructive in Italy. Last summer a serious infestation of leaf beetles occurred in the EEC experimental field so I had the chance to assess visually the severity of attack and to discover the existence of ample variability in the susceptibility among elm species and clones. The highest susceptibility is shown by *U. carpinifolia* and *U. pumila*, the lowest by *U. wilsoniana* and *U. parvifolia*, with *U. japonica* taking an intermediate or low place.

The use of elm species resistant to Dutch elm disease

Our breeding programme relies mainly on the three species which in the literature are regarded as more resistant, even if the material to hand is scanty. *U. pumila* is well-suited to the Italian climate, grows fast and carries a high level of resistance to DED. Of this species, we have been using some individuals found growing in Italy, some of them probably natural hybrids with *U. carpinifolia*. Other combinations will be made using the selections included in the EEC project. The Siberian elm *U. pumila* presents two drawbacks: an aesthetically displeasing shape and high susceptibility to the leaf beetles.

U. parvifolia, which is generally a species of moderate growth rate, includes some individuals which exhibit rapid growth. This is the case of a clone (NA36533) selected by Santamour in Washington, D.C. In addition it exhibits rather wide leaves, only a little smaller than those of *U. carpinifolia*, and a very high level of resistance to DED as well as to *Galerucella*. Drawbacks of this clone are a serious lack of wind-hardiness and also a lack of crown symmetry. I have been trying for two years to cross this clone with *U. carpinifolia* by preserving the pollen of the latter species at 5°C and 10 per

cent relative humidity from February until September, but so far unsuccessfully. I will try again using *U. parvifolia* as the male parent. Other provenances of *U. parvifolia* from China and Japan are growing in Florence but are not in the reproductive stage, so it is too early to estimate the possibilities offered by the Chinese elm.

I have only one genotype of the third more resistant species, *U. wilsoniana*, and this is the clone 3–14 selected by Schreiber and Townsend at Delaware, Ohio. The clone originated in Arnold Arboretum, Massachusetts, U.S.A. from open pollinated seed collected from the single clone of *U. wilsoniana* present; it may thus well be a hybrid between *U. wilsoniana* and some other elm species. The resistance level to DED is good and that to *Galerucella* is very good. It has a nice leaf, fast growth, open-crown shape with the main branches hanging slightly in the first years. I obtained some cross-combinations but I need some more provenances in order to explore the potential of this species.

Other species of elm with a lower but interesting level of resistance to DED are *U. wallichiana*, which is present in many of the Dutch selections. *U. japonica*, fairly resistant to the leaf beetles, *U. laciniata* and *U. villosa*. Some of them are present in hybrids selected by Heybroek, Smalley and Santamour and can be used for more complex cross-combinations.

Two selections of *U. japonica* already released in Canada, two provenances of *U. laciniata*, and one clone of *U. villosa* should enter the EEC adaptability test and I have requested this material for the purpose of breeding. So far my request has not been fulfilled because the propagation techniques have not been entirely successful. I would stress the importance of testing this material in Italy, at least, where *U. villosa* in partiular would thrive and would represent a valuable addition to my elm breeding work. In order to speed up the work in progress I am asking that new genetical material introduced in Europe under the EEC project should quickly be made available to collaborators who work in the field.

As for the mating design I planned my work in accordance with the North Carolina Model 2 which allows one to estimate heritability, general and special combining abilities and genetical gain for resistance. The male parents are three clones of *U. carpinifolia*, three clones of *U. pumila* and one clone of *U. wilsoniana*. But as the work proceeds I am compelled for several reasons to use more male parents so I am adding more cross-combinations to my mating design. I am now in the stage of inoculating and selecting the first progenies coming from the breeding work.

The status of Dutch elm disease in Italy

From a survey carried out in 1978 I noticed that the disease normally stopped in the mountain zones at an elevation of 800–900 m. But because in at least one case DED was found at the highest altitude at which elm lives, I anticipated that it was only a matter of time before those areas were reached by the disease. In fact in 1981 DED was found in Tuscany at 1000 m elevation in the Arboretum of Vallombrosa and in the forest of Camaldoli, and at 1100 m at La Verna. The aggressive strain has also been isolated at the highest elevations.

Another interesting datum which came from the survey was that the severity of the disease was decreasing in the southern regions of Italy where the non-aggressive strain was more frequently isolated, and the great part of Calabria was free from the disease. I noted that the scanty population of *U. carpinifolia* in Algeria appeared to be still untouched by DED during my visit in the summer of 1981. Considering this information with respect to laboratory data by Brasier, Lea and Rawlings (1981) regarding the diversity of temperature requirements of the aggressive and non-aggressive strains of *C. ulmi*, it is possible that the aggressive strain would encounter environmental constraints in a warmer and drier climate. But information over the last year has revealed a notable southwards shift of the highest intensity attacks suggesting that the disease entered the country from the north and moved towards the south.

Moreover last September I took samples from an epidemic outbreak in Sardinia (provinces of Sassari and Oristano) and found that the majority of isolates belonged to the aggressive strain. So, it seems to me that the possibility that climate can significantly alter the ratio of the *C. ulmi* strains in southern Italy is minimal. However it is important to check more precisely this possibility, which I will do shortly by sampling a southern area in Sicily or Calabria.

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PAPER 20

Resistant elms for Europe

H. M. HEYBROEK

'Dorschkamp' Research Institute for Forestry and Landscape Planning, Wageningen, The Netherlands

Introduction

The appearance of the aggressive strain of *Ceratocystis ulmi* has enhanced fears that the elm might largely be wiped out as a useful tree in Europe, like the chestnut was in North America. Such a loss could be measured in terms of the costs of the removal and replacement of these trees, or in terms of their lost functions of providing shelter and a valued timber, and of giving beauty and variety to landscape, gardens, parks and cities. It could also be described as a cultural loss for mankind. For over five thousand years and in various capacities, the elm has been a close companion of European man and a significant part of his environment. We cannot expand on the cultural history of the elm here, but the fact that the elm was more than "just a tree" to our ancestors, that to them it had a certain personality, is exemplified in the Germanic myth that the first woman was created from an elm log. There is every reason not to give up on this tree.

In spite of improved methods for the control of Dutch elm disease (DED), it still seems that in the long run the future of the elm in Europe depends on resistant selections. The European Economic Community (EEC) research project on Dutch elm disease therefore rightly devoted a good deal of its effort to breeding and to resistance studies. This paper will survey the accomplishments up to now, the present state of the art, and future developments and goals.

The Dutch elm breeding programme

When summarizing the results of the Dutch elm breeding programme, it is convenient to distinguish three groups or "generations" of clones.

The first "generation" consisted of the clones 'C. Buisman' (released 1936) and 'Bea Schwarz' (1947). Both were selected from populations of seedlings, obtained from the wild or from a seed merchant, in Spain and France respectively. Neither satisfied; both have disappeared from the trade in the Netherlands, as they were generally disappointing with reference to growth rate, shape and foliage. In addition, the 'Buisman' elm always suffered from *Nectria cinnabarina* (Tode) Fr. so that each tree became damaged to some extent. Some city foresters think that the 'Bea Schwarz' elm, if trained well when young, can make a decent small tree that needs little maintenance and that can be useful in certain locations where a tree with a big crown would not fit. No new plantings are made as its resistance is not sufficient against the aggressive strain of *C. ulmi*.

The second "generation" consisted of two F1 hybrids from controlled crosses, 'Commelin' (1960) and 'Groeneveld' (1963). Both were planted on a large scale in the Netherlands: according to figures of the General Inspection Service for Arboriculture (NAKB) 640,000 and 289,000 trees, mainly grafts, of these clones were sold to end-users up to 1980. Though exports and some losses account for a small proportion of the totals, the large majority of those trees were planted in the towns and countryside of the Netherlands.

Because of the clone's susceptibility to the aggressive strain of *C. ulmi* sales of 'Commelin' are down and close to nil now: 5,000 in 1980/81 compared to 126,000 in 1972/73. Following artificial inoculation the trees often die back to ground level. In the field, under the Dutch conditions of strict sanitation, losses to DED are low up to now, but so are the losses in the 'Vegeta' elm that was planted on a similar scale in the same period. We advise strongly against new planting, which is considered too risky.

The Groeneveld elm, at the time of its release (1963), was seen as a by-product of the breeding programme, as a small tree for special city use (Heybroek, 1964). Interest in it has increased, however, as its level of resistance to DED is sufficiently high for restricted planting in spite of the presence of the aggressive strain. It will not always survive an infection by that strain, but losses are expected to be an expection rather than the rule.

Table 1. Average sizes of elm clones in different trial plots in the Netherlands.

Elm clones \ Trial plots	Polders N 65 age: 1+16			Geestmer-ambacht age: 1+11			Bleiswijk age: 1+10			Polders Lepelaarsweg age: 3+8			Brielle age: 2+7			Polders F 52 age: 1+7		
	h̄ m	d̄ cm	n	h̄ m	d̄ cm	n	h̄ m	d̄ cm	n	h̄ m	d̄ cm	n	h̄ m	d̄ cm	n	h̄ m	d̄ cm	n
Vegeta	16.3	23.6	8													7.0	9.6	45
Commelin	15.9	22.2	24	12.4	22.7	8	10.5	16.6	18	6.0	8.5	88	9.4	13.9	6	7.6	9.7	45
Groeneveld	14.2	18.9	24	11.0	20.3	8	9.4	15.9	11									
Lobel				12.7	24.4	8	9.3	16.4	13	5.5	8.2	9	9.5	15.6	11			
Plantyn				12.7	23.0	8	9.4	15.9	10	4.8	6.4	8	9.0	12.7	11	6.6	9.5	45
Dodoens				12.6	25.0	8	9.6	20.2	14	4.7	7.7	9	9.3	16.0	11	6.6	8.4	45
Clusius				11.7	26.0	8	10.1	19.2	4	6.1	11.8	8	9.0	14.7	9			

Age = (age from grafting at planting) + (age from planting at moment of measuring).
h = height. d = diameter at breast height. n = number of trees measured.

Growth of the one- and two-year old grafts is relatively slow, so that we anticipated it would never grow to be a big tree. Maybe it will not, it is too early to say; but after that slow start its growth is better than expected, as we can see in many trial plots (Table 1). We still recommend it should be planted on good sites only. The tree will sometimes flower superabundantly, most of the buds being flowerbuds. After the big crop of fruits is shed, the crown is thin and partly bare, and it is midsummer before the crown recovers and bears sufficient leaves to look aesthetically pleasant. We fear that on a poor site flowering might be stronger and, even worse, recovery might be too slow.

The third "generation" elm clones, released in 1973 after the aggressive strain was recognised, consisted of the clones 'Lobel', 'Dodoens' and 'Plantyn'. The three had different (European) fathers, but a common mother: clone 202, which in its turn derived from the controlled cross *U. wallichiana* (from India) x *glabra* 'Exoniensis' (from England). Their resistance to DED is comparable to that in 'Groeneveld': it seems acceptable without being fully satisfactory. In spite of their relatively high level of resistance, it remains possible that the disease might kill an individual tree, but we expect losses will be low when expressed as a percentage of the population. This should be taken into consideration when plantings are made. It seems unwise to use the clones for large formal plantings, where an occasional killed tree leaves a conspicuous gap, ruining the rhythm of the design. They should rather be used in informal or mixed plantings in which a live tree is enjoyed but a dead one not excessively missed.

The clones have an impressive set of good properties. Their growth is fast (Table 1), faster than 'Belgica' on several soils. The foliage is dark green and healthy, deep into autumn, though the leaves of 'Plantyn' may curl up temporarily during a drought period. They have rarely be found attacked by *Nectria cinnabarina*, and they are not very sensitive to wind. 'Lobel' especially has been seen to grow unaffected under conditions of considerable exposure to sea wind.

The three clones are visibly related when it comes to shape. Branching is more or less fastigiate. 'Lobel' is the narrowest of the three with a columnar crown; 'Plantyn' has the widest crown. The crowns might get wider with age, as is the case in their common grandparent *U. glabra* 'Exoniensis': this clone is narrow columnar when young, but tends to be globular in shape or even wider when mature. Like most elms, the three need some pruning in their formative years as a help to develop a good crown.

A new clone

Preparations are under way to release a new clone to the trade under the name 'Clusius' (Figure 1). In many trials it is represented under the number 568. It has the same parents as 'Lobel' and belongs in all respects to the group that was described above as the "third generation". The differences are small, but significant for some purposes. Its resistance to DED is marginally better or at least at the same level as in the other three. Leaves are larger and of a fresher green than in 'Lobel', the shape of the crown is somewhat different if not better, resistance to wind is high, diameter growth is better. We have to await its further development, but I am convinced it will prove its worth next to the others. It is valuable to have some more variation within the same theme.

The clone is named after Carolus Clusius (or Jules-Charles de l'Escluse), one of the "fraternal triumvirate" of botanists and herbalists in the Low Countries in the 16th century. The other two

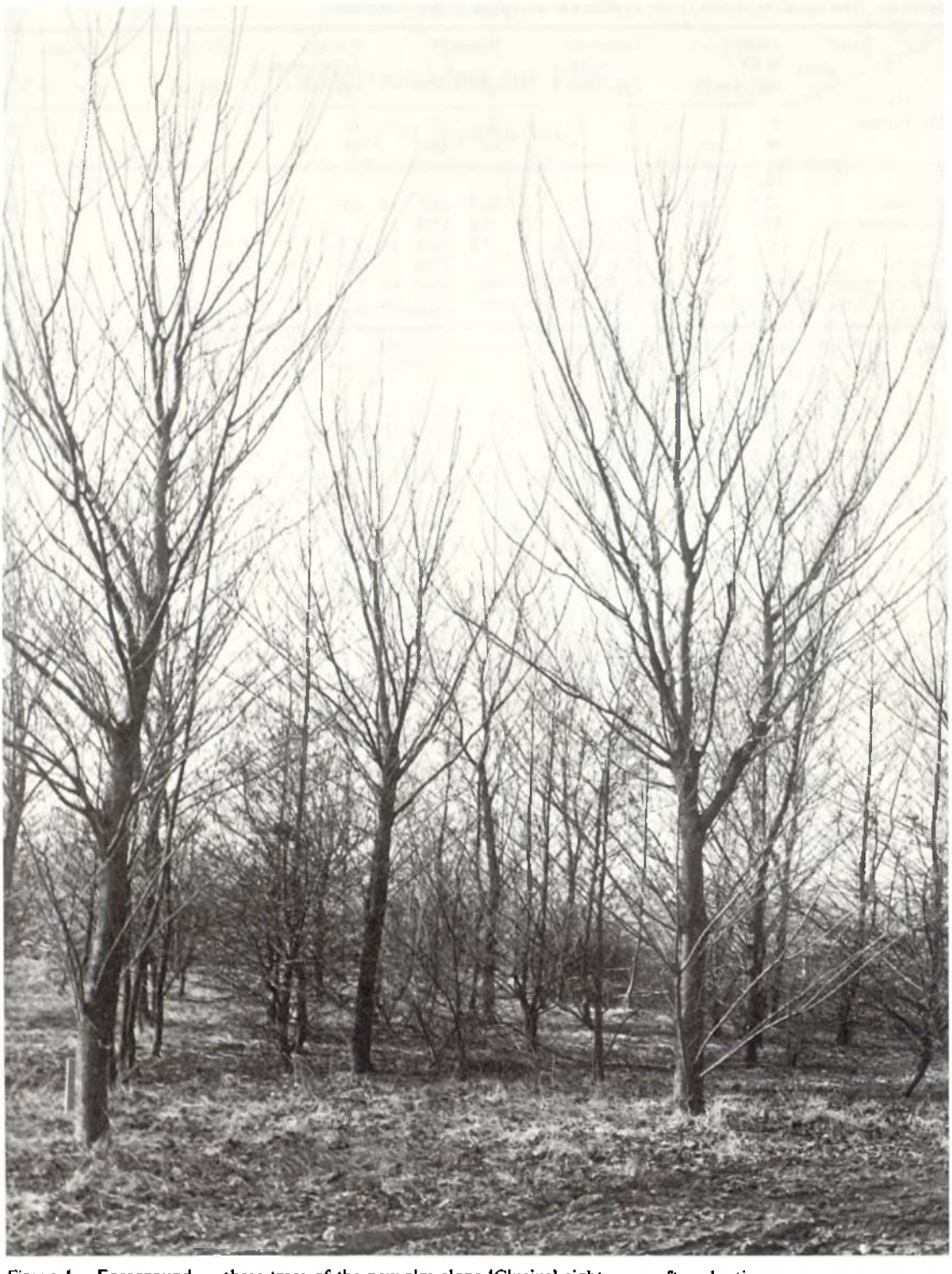

Figure 1. Foreground — three trees of the new elm clone 'Clusius' eight years after planting.

were Rembert Dodoens and Mathias de Lobel. Clusius was a great and erudite European, speaking eight languages fluently, working as a botanist and mycologist in Spain, Portugal, France, Austria, England and Holland. He introduced the potato to the Continent, and founded the culture of flower bulbs in the Netherlands. We can only hope the clone will live up to that name.

The fourth "generation"

It has rightly been remarked that the arrival of the aggressive strain has caused a revolution in the Dutch elm breeding programme (Brasier, 1982). First the considerable collection of existing experimental clones and seedlings was screened over the much wider sieve of the aggressive strain: many were discarded, others were kept for reference or breeding only. Many new clones were made of promising seedlings. New crosses were made too, combining parents with the highest resistance, putting a renewed emphasis on Asiatic elms. However, a conscious effort was also made to attain through various combinations maximal resistance in elms of a purely European background. A breeder should try several roads to success concurrently.

The new strain, necessitating a much sharper selection, severely reduced the available pool of resistance genes. This is exemplified by the fact that the three-plus-one most recent releases are so narrowly related. A broadening of the basis of the breeding population was needed. For several reasons, we chose to concentrate on fresh Asiatic imports. A report on the present status of that part of the programme will appear elsewhere (Heybroek, 1982). The latter approach was not devised or expected to produce results in the form of ready-to-use resistant clones or populations.

Elms for the future

Aims of the Dutch elm breeding programme

These research efforts are beginning to show up in the form of promising experimental clones. However, none is ready for release yet. We thus cannot yet describe this "fourth generation", as we dubbed it here. We can only describe the goals we hope to realize in this material. They are threefold:

1. A higher level of resistance. This is the first requirement. It is true that absolute resistance to DED does not seem to exist in elms, and it seems unrealistic to require that all new varieties should be able to remain fully unscathed when attacked by excessive numbers of spore-carrying elm bark beetles. A certain measure of sanitation is regarded as normal management in the silviculture of several conifers from protection against their bark beetles, and it is not unreasonable to require some such precautions for the safe growing of elms. The trees should not, however, succumb under the attack of smaller, more normal populations of the beetles. Presumably, elms with a high level of resistance to DED will again be able to recover from an attack, as many of the susceptible English elms used to do when infected with the non-aggressive strain (Peace, 1960). We do see such recovery in grafts of the more resistant clones in the year after inoculation with the aggressive strain.
2. A wider variation in tree shapes. The "third generation" covers a narrow range of shapes; in addition, we certainly want trees with a wider branching angle, a broader crown, if possible with the ease and grace of twigs and foliage such as we find in the Belgica elm. A wide range in shapes and properties is needed.
3. A wider variation in resistance genes. We do not expect another major increase in pathogenicity of *C. ulmi* above that caused by the introduction and spread of the aggressive strain. Even so, it seems unwise to depend so largely on a few narrowly related clones. Resistant elms with a different genetic background are needed for reasons of diversification.

We have additional goals and wishes. We certainly want to develop seedling populations with sufficient resistance and other good properties to be planted out as a population, but we are not yet able to produce them. We should like to use or incorporate any resistance or unattractiveness to the elm bark beetles, but this property seems to be rare and not yet easily assessed. On an experimental scale, we will investigate the potentials of crosses with *U. villosa*, of polyploidy and of artificial mutations. There is some demand for certain ornamental garden elms.

Results of other breeding programmes

More and more clones are being released in North America; some of them are being planted in Europe. Among them are 'Sapporo Autumn Gold' and 'Regal', developed at the University of Wisconsin in Madison, Wisconsin, the 'Urban' elm of the Nursery Crops Laboratory, USDA, in Delaware, Ohio; the 'Thomson' elm of the Tree Nursery, PFRA, Indian Head, Saskatchewan; and 'Jacan' and 'Mitsui Centennial' of Morden Experiment Station, Manitoba. Others might follow soon, including an interesting hybrid with *U. parvifolia* made in the National Arboretum at Washington, DC.

Table 2. Identity of clones planted in the two trials laid out in 1979/80 and 1980/81.

Name	Number	Parentage (1)	In trial of 1979/80	In trial of 1980/81
'Groeneveld'	296	*glabra* 49 x *carpinifolia* 1	+	+
'Lobel'	454	202 (2) x 336 (Bea Schwarz selfed)	+	+
'Dodoens'	494	202 open pollinated	+	+
'Plantyn'	496	202 x 302 (*carpinifolia* 1 x *carpinifolia* 28)	+	+
'Clusius'	568	202 x 336	+	+
'Vegeta'	P38	(seedling England *ca.* 1750)	+	
'Urban'	P607	148 (Vegeta x *carpinifolia* 28) x *pumila*	+	
'Sapporo A.G.'	P614	*pumila* x *japonica*	+	
—	502	215 (*pumila* x Hoersholmiensis) x Commelin		+
—	506	Groeneveld x 215		+
—	507	Vegeta x Groeneveld	+	
—	610	215 (*pumila* x Hoersholmiensis) x Groeneveld	+	+
—	631	Plantyn x 495 (148 x 297 (3))		+
—	637	Plantyn x 495		+
—	691	202 x 336		+
—	808	543 (202 x *carpinifolia* 1) open pollinated		+
—	818	Dodoens open pollinated		+
—	822	Dodoens open pollinated		+
—	846	Hoersholmiensis open pollinated		+
—	884	248 (*wallichiana* x *carpinifolia* 1) x 215		+
—	906	215 x 125 (Vegeta x *carpinifolia* 1)		+

(1) parentage of parents given at first listing only.
(2) 202 = *U. glabra* 'Exoniensis' x *wallichiana* P39.
(3) 297 = *U. carpinifolia* 'Christine Buisman' open pollinated.

Some of these may prove to be useful for us. Being developed for different uses in a different climate, however, they need to be tested before we can assess their value under our conditions. Trees that have *U. pumila* as one parent have often shown to be not fully adjusted to the West European climate. The Canadian clones of *U. japonica*, which are admirably hardy in the prairie provinces, might grow slower than we like. It is quite possible, however, that testing will show that a particular clone can be quite useful in some part of Europe.

The function of the European trial plots

Since, 1980, trials with various new elm clones have been laid out in the different countries of the EEC. Plants were grown in Wageningen, transported by car and planted out in trials of a uniform design. The materials involved are specified in Table 2. They include the new clone 'Clusius'. The same range of clones is also present in Madison, Wisconsin.

The functions of these plantings are fivefold:

1. The trials should demonstrate locally the growth and development of released clones in comparison to a known standard such as 'Vegeta'. This alone may well speed up the introduction of these clones in the planting practice of a country by ten years or more. Further important observations can further be made about characters of a qualitative nature such as crown shape, foliage, phenology, attack by certain pests, frost damage, etc. Conclusions on the more quantitative characters such as growth rate should be made with caution, remembering that a single trial can give no more than a limited indication of a clone's reaction pattern in a large country. If the clone does very well, that is a good sign; if it does poorly, it may be wise to try it on a different site.

   The plants can also be used to provide propagating material for experiments, as long as this does not harm the trees' growth or shape which we want to observe and compare.

   The trials are not really designed to provide information about the trees' resistance to DED. The numbers of trees are too small for that, and the natural infection process too erratic. Better comparisons can be made using artificial inoculations in the nursery.
2. The trials can play a big role in producing the "fourth generation", in identifying superior clones that should be released. The trials contain many new and experimental clones that are considered promising in some respect, and the network of plantings under various conditions will allow an earlier decision to be formulated about a clone's value and its possible release.
3. The trials can give some first indications about gene-environment interaction. A tree that is

Table 3. Provenance of populations of elm obtained from Japan in the two trials laid out in 1979/80 and 1980/81. Population 73 p is *U. pumila* and introduced, the others are *U. japonica* and native.

Population number	Location	Prefecture	Co-ordinates	Height above sea level (m)	In trial of 1979/80	In trial of 1980/81
2 p	Akagi	Kumamoto	32° 45′ 131° 10′	700	+	
3 p	Akagi	Kumamoto	32° 45′ 131° 10′	650	+	
57 p	Kuru midai	Akita	39° 50′ 140° 38′	350	+	
58 p	Kuru midai	Akita	39° 50′ 140° 38′	350	+	
59 p	Kuru midai	Akita	39° 50′ 140° 38′	350	+	
73 p	Sapporo Botanic Garden				+	
90 p	Niikappu	Hokkaido	42° 36′ 142° 26′	130	+	
23 p	Syobu-gahama	Gunma	36° 45′ 139° 24′	1200		+
28 p	Senzyo-gahara	Gunma	36° 45′ 139° 24′	1200		+
41 p	Oze-gahara	Gunma	36° 55′ 139° 15′	1400		+
46 p	Oze-gahara	Gunma	36° 55′ 139° 15′	1400		+
47 p	Iubiso	Gunma	36° 48′ 138° 58′	620		+
78 p	Sapporo	Hokkaido	43° 04′ 141° 21′	50		+
93 p	Niikappu	Hokkaido	42° 36′ 142° 26′	80		+
95 p	Niikappu	Hokkaido	42° 36′ 142° 26′	130		+
127 p	Nagano	Nagano	36° 41′ 138° 11′	1200		+

superior (in comparison with a standard clone) at one location may be inferior at a different site. Some minor interactions of that sort can even be found in Table 1; one can expect them to be larger in a range that extends from Aars to Firenze and from New Ross to Bad Sooden-Allendorf. Some minor disease or pest may be absent in one country, serious in another. Though the interpretation of this information requires caution, it may give important indications both for local advisory work and for future selection and breeding. It might lead to new selective introductions out of the Dutch gene pool. For the same reasons, some seedling populations of various provenances of Japanese elm (*Ulmus japonica*, syn. *U. davidiana*) and *U. pumila* have been added, to see how widely adapted these materials are (Table 3).

4. The elm is needed for different purposes in different countries: the "ideal" elm looks different in each. In combination with the third point, this means that a clone which looks mediocre and scarcely desirable in Holland, so that no one would plan to release it, might well be perfect for some purpose in another country and thus should be released for that use. The EEC research programme, making this possible, allows that more fruits are reaped from the original heavy Dutch investment in the breeding programme.
5. If gene-environment interactions are strong, and if conditions and requirements appear to deviate too much from those in the Netherlands where the basic breeding work is carried out, this might lead to the decision to start a programme of introduction, selection or breeding of elms locally, building on the materials in the trials and on the Dutch experience. This happy outcome seems to be true for Italy at present.

Materials for a third trial are being prepared in Wageningen, to be planted in 1982/83. In combination with the two earlier trials, it will help to give a new future to the elm in Europe.

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Enquiries relating to this publication should be addressed to
the Publications Officer, Forestry Commission Research Station,
Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH

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