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Phytophthora Disease of Alder in Europe

Edited by John Gibbs, Cees van Dijk and Joan Webber

Forestry Commission

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Preface

This Forestry Commission Bulletin is the principal output of Concerted Action FAIR5 CT97 3615 that began on March 1st 1998 and finished on February 28th 2001. Scientists from fourteen partner organisations in eleven countries took part in the Concerted Action and they were joined by other scientists for some of the workshops.

The objectives were to draw together information on the current and potential impact of the Phytophthora disease of alder in order:

1. To determine if the spread of the disease within Europe can be limited.
2. To make recommendations on disease management and control.
3. To identify research requirements.

These issues are addressed in the Bulletin, and it is thought they will prove to be of interest to many. Also of interest is the fact that the work on this disease has thrown up information of very considerable importance on the nature of plant pathogens. The discovery that the 'alder *Phytophthora*' is a hybrid between two species of *Phytophthora*, neither of which has the capacity to attack alder and that one or both parents are probably introduced to Europe, is of enormous significance. The point was addressed by C. M. Brasier in the editorial section of the journal *Nature* (The rise of the hybrid fungi, *Nature* 405, 134–135, 2000). The key issues were summarised in a separate paper which was submitted to the Standing Committee on Plant Health of the European Union.

Various colleagues in the Concerted Action took responsibility for different areas of work and the chapters of the Bulletin reflect this. We, the editors, have been responsible for the final product.

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Summary

The current status of the host and the disease

Alders play a vitally important role in Europe. They have a diversity of characters which not only enables them to establish as pioneers but also allows them, at least in the case of common alder *Alnus glutinosa*, to play a significant part in climax forests. *A. glutinosa* also makes a major contribution to the ecology and stability of river banks. All four European alder species are important in the establishment of woodland on difficult sites.

Apart from the Phytophthora disease, European alders are largely free from major pest and disease problems. Certain stem-boring insects can cause quite conspicuous dieback and drought has often been implicated as a cause of damage. Other 'diebacks' of unknown cause have occurred from time to time. However, a critical analysis of the literature has largely disposed of the idea that the Phytophthora disease might have been present long before it was recognised in the UK in 1993. It now seems unlikely that it has been in existence for much more than 30 years. The key symptom is a necrotic lesion in the inner bark of the stem and this lesion is often marked externally by the production of a tarry or rusty exudate. In severely affected trees, the foliage is small, sparse and often yellowish. Heavy fruiting commonly occurs. Such trees normally die quite rapidly but some can recover.

One particular *Phytophthora* species – known as the 'alder Phytophthora' – is associated with the vast majority of cases and has been shown to be highly pathogenic to alder. It is of hybrid origin – the parents being *P. cambivora* and a fungus close to *P. fragariae*. Neither of these fungi is thought to be native to Europe, both probably having been introduced during the course of trade in plants or plant products. The 'alder Phytophthora' is therefore likely to owe its origin to man's activities. It is now known to consist of a hybrid swarm and so the term 'alder Phytophthoras' is sometimes used. The components of the swarm comprise the widely distributed 'standard type' and a number of 'variants', some of which seem to have a restricted distribution. The variants may be genetic breakdown products of the standard type or they may be the products of backcrosses or of further hybridisation events. Some of the variants are less pathogenic than the standard type.

The disease has now been recorded in eleven countries: Austria, Belgium, France, Germany, Hungary, Ireland, Italy, Lithuania, the Netherlands, Sweden and the UK. Very high losses have occurred in some localities while in others the disease impact has been small. Some riparian survey plots show a rapid and inexorable increase in mortality (for example, in the south of the UK and in northeastern France) while others (e.g. in upper Austria) show a notable recovery from disease. In one place, namely the natural wetland of Die Wieden in the Netherlands, a pathogenic variant of the fungus is present without apparently causing any detectable damage. Little is known about the influence of host vigour on disease development, but this may be a factor in the sudden appearance of disease in two streams in the southern part of the Netherlands following the artificial raising of water levels.

The possibility of limiting pathogen spread

The importance of rivers and other watercourses in the dissemination of the pathogen is well established, and, once the pathogen is present in a river system, downstream dispersal is inevitable. There is strong evidence that alders from nurseries have played a major role in the introduction of the pathogen to new areas. To minimise further long-distance dissemination of the pathogen by this

means, Partners in the Concerted Action recommend that strict phytosanitary regulations should be imposed on plants entering disease-free countries in Europe and elsewhere in the Northern Hemisphere. Also they propose that a certification scheme should be developed for alder plants from the nursery once molecular detection techniques have been developed. Until then it is recommended that a Code of Good Practice should be developed. Inter alia this would require that there is no river irrigation of the nursery, that there is a health inspection of alder plants during the growing season and that there is routine disinfection of the nursery between crops.

Options for disease management and control

Studies on the coppicing of affected trees are at an early stage and little is known about the disease levels that can be expected in the new growth. Chemical treatments remain untried but probably offer little scope for use. As yet, studies on host resistance have given disappointing results, suggesting that resistance genes may be rare and that a long-term resistance screening programme will be required. For such work, the diversity and instability in the causal pathogen will need to be borne in mind.

Future research needs

Under a Concerted Action, research can only occur as the funding of the partner organisations permits. The Partners identified the following key priorities for future work:

- Develop molecular tools for the identification of alder Phytophthoras in soil, water and host tissue.
- Understand the infection process, including survival of the pathogen and propagule dissemination.
- Understand factors influencing host invasion, with special reference to the influence of environmental factors on the ability of the host to resist the effects of the pathogen.
- Extend knowledge of the diversity and stability of the alder Phytophthora genotypes found across Europe.
- Establish and maintain monitoring plots to enable disease development to be modelled and predicted.
- Screen for resistance to the pathogen, taking particular note of trees showing freedom from disease in areas of high mortality.
- Investigate the role of nurseries in disease dissemination.
- Develop disease control strategies in nurseries.

Résumé

Le point sur l'hôte et la maladie

Les aulnes jouent un rôle d'une importance vitale en Europe. Ils présentent une diversité de caractères qui leur permettent non seulement de s'établir en pionniers mais aussi, au moins dans le cas de l'aulne glutineux *Alnus glutinosa*, de jouer un rôle important dans les forêts climax. *A. glutinosa* apporte aussi une contribution majeure à l'écologie et à la stabilité des berges des rivières. Les quatre espèces d'aulnes spontanées en Europe sont toutes importantes à l'établissement des bois sur des sites difficiles.

Mis à part la maladie du Phytophthora, les aulnes européens sont dans une large mesure exempts de problèmes majeurs causés par les ravageurs et les maladies. Certains insectes creusant des galeries dans le tronc peuvent causer un dépérissement notable et la sécheresse se trouve souvent impliquée comme cause de dégâts. D'autres "dépérissements" dont les causes ne sont pas connues ont aussi été constatés de temps à autre. Néanmoins, l'analyse critique de la documentation a en grande partie éliminé l'idée que la maladie du Phytophthora aurait été présente longtemps avant d'avoir été identifiée au Royaume-Uni en 1993. Il semble maintenant peu vraisemblable qu'elle existe depuis beaucoup plus de trente ans. Le symptôme-clé est la présence d'une lésion nécrotique de l'écorce interne du tronc et cette lésion se manifeste souvent extérieurement par l'apparition d'un exsudat goudronneux ou brun roux. Chez les arbres sévèrement touchés, les feuilles sont petites, peu nombreuses et souvent jaunâtres. Il arrive fréquemment que la fructification soit très abondante. Les arbres meurent normalement assez rapidement, mais certains peuvent guérir.

Une espèce particulière de *Phytophthora* – nommée le 'Phytophthora de l'aulne' – est associée à la grande majorité de ces cas et s'est avérée très pathogénique pour l'aulne. C'est une espèce d'origine hybride – ses parents étant *P. cambivora* et un champignon proche de *P. fragariae*. Aucun de ces champignons n'étant censé être originaire d'Europe, ils ont probablement été introduits tous les deux au cours du commerce des plantes et des produits végétaux. Les activités de l'homme sont donc vraisemblablement à l'origine de l'apparition du 'Phytophthora de l'aulne'. On sait maintenant qu'il consiste en un essaim hybride et le terme de 'Phytophthoras de l'aulne' est donc parfois utilisé. Les éléments de cet essaim comprennent le 'type standard', très répandu et un 'nombre de variantes', dont certaines semblent avoir une distribution restreinte. Les variantes peuvent être les produits de la décomposition génétique du type standard ou les produits de croisements en retour ou d'autres cas d'hybridation. Certaines variantes sont moins pathogéniques que le type standard.

La maladie a été identifiée dans onze pays: l'Autriche, la Belgique, la France, l'Allemagne, la Hongrie, l'Irlande, l'Italie, la Lituanie, les Pays-Bas, la Suède et le Royaume-Uni. Dans certaines régions les pertes ont été très sévères, tandis que dans d'autres l'impact de la maladie a été réduit. Quelques parcelles ripicoles étudiées montrent un accroissement aussi rapide qu'inexorable de la mortalité (par exemple dans le sud du Royaume-Uni et dans le nord-est de la France), tandis que d'autres (par exemple en Haute-Autriche) montrent un taux de guérison notable. Dans un endroit, à savoir la zone humide naturelle de Die Wieden aux Pays-Bas, on note la présence d'une variante pathogénique du champignon sans que celle-ci ne cause apparemment de dégâts détectables. On sait peu de chose à propos de l'influence que peut avoir la vigueur de l'hôte sur le développement de la maladie, mais ce pourrait être un facteur de l'apparition soudaine de la maladie constatée dans deux cours d'eau de la partie sud des Pays-Bas, à la suite de l'élévation artificielle des niveaux d'eau.

La possibilité de limiter la propagation du pathogène

L'importance des rivières et autres cours d'eau dans la dissémination du pathogène est bien établie, et, une fois que le pathogène est présent dans un système fluvial, sa dispersion en aval est inévitable. Tout semble indiquer que les aulnes provenant de pépinières ont joué un rôle majeur dans l'introduction du pathogène dans des zones nouvelles. Pour minimiser toute autre dissémination à longue distance du pathogène par ce moyen, les Partenaires de cette Action Concertée recommandent qu'une réglementation phytosanitaire rigoureuse soit imposée sur les plants entrant dans les pays d'Europe non touchés par la maladie, ainsi qu'ailleurs dans l'Hémisphère Nord. Ils proposent aussi qu'un système de certification soit développé pour les plants d'aulnes des pépinières une fois que des techniques de détection moléculaire auront été développées. En attendant il est recommandé qu'un Code de Bonne Pratique soit développé. Entre autres ceci exigerait que la pépinière ne soit pas irriguée par un cours d'eau, que l'on procède à une inspection de santé des plants d'aulne pendant la saison de croissance, et que la pépinière soit systématiquement désinfectée entre les cultures.

Options pour la gestion et la lutte contre la maladie

Les études effectuées sur l'élagage des arbres touchés en sont à leurs débuts et l'on sait peu de choses sur les niveaux de maladie que l'on pourrait s'attendre à trouver sur la nouvelle pousse. Les traitements chimiques restent non-testés mais offrent probablement peu de possibilités d'utilisation. Jusqu'ici les études effectuées sur la résistance de l'hôte ont donné des résultats décevants, suggérant que les gènes de résistance seraient rares et qu'un programme de tests de résistance à long terme sera nécessaire. De tels travaux exigeront que l'on tienne compte de la diversité et de l'instabilité du pathogène causal.

Besoins de la recherche à venir

Dans le cadre d'une Action Concertée, la recherche n'est possible que si le financement fourni par les organisations partenaires le permet. Les partenaires ont identifiés les priorités-clés suivantes pour les travaux à venir:

- Développer des outils moléculaires pour l'identification des Phytophthoras de l'aulne dans le sol, l'eau et le tissu hôte.
- Comprendre le processus de l'infection, y compris la survie du pathogène et la dissémination des propagules.
- Comprendre les facteurs influençant l'invasion de l'hôte, en tenant spécialement compte de l'influence des facteurs environnementaux sur la capacité de l'hôte à résister aux effets du pathogène.
- Etendre les connaissances sur la diversité et la stabilité des génotypes de Phytophthora de l'aulne trouvés à travers l'Europe.
- Etablir et entretenir des parcelles de surveillance pour permettre de modéliser et prédire le développement de la maladie.
- Procéder à des tests de résistance au pathogène, en attachant un intérêt particulier aux arbres non touchés par la maladie dans les zones de forte mortalité.
- Etudier le rôle joué par les pépinières dans la dissémination de la maladie.
- Mettre au point des stratégies pour enrayer la maladie dans les pépinières.

Zusammenfassung

Gegenwärtiger Kenntnisstand über die Wirtsbaumarten und den Krankheitserreger

Erlen wird eine wichtige Funktion in europäischen Wald- und Gewässerökosystemen zuteil. Sie weisen eine Vielzahl von Eigenschaften auf, die sie nicht nur befähigen, als Pionierpflanzen neue Flächen zu besiedeln, vielmehr, wie im Falle der Schwarzerle (*Alnus glutinosa*), spielen sie auch eine signifikante Rolle in Wald-Klimaxgesellschaften. Ebenso hat *A. glutinosa* einen wichtigen Anteil an der Ökologie von Fließgewässern und trägt zur Stabilität von Flußufern bei. Alle vier in Europa vorkommenden Erlen-Arten sind für die Etablierung von Wäldern auf problematischem Gelände von großer Bedeutung.

Abgesehen von der hier beschriebenen Wurzelhalsfäule, die von pilzlichen *Phytophthora*-Erregern verursacht wird, sind ansonsten nur wenige Probleme mit Schädlingen und anderen Krankheiten an Erlen bekannt. So kann ein Befall mit den Stamm zerstörenden Insektenarten ein weites Absterben des ganzen Baumes zur Folge haben. Auch werden oft Dürre und Trockenheit als Schadensursache für ein Erlensterben verantwortlich gemacht. Und hin und wieder wird von einem Absterben von Erlen ohne ersichtliche Ursache berichtet. Die Idee, daß die Phytophthora-Krankheit schon früher, lange, bevor sie erstmals im Jahre 1993 in Großbritannien beschrieben wurde, in Europa vorgekommen ist – wenngleich auch als solche unerkannt – muß nach eingehender Untersuchung der Literatur allerdings verworfen werden. Es scheint jetzt eher unwahrscheinlich, daß die Krankheit bereits wesentlich länger als 30 Jahre besteht. Das Krankheitsbild ist durch das Auftreten von exsudierenden Rindennekrosen gekennzeichnet. Hierbei sterben im unteren Teil des Stammes weite Bereiche der inneren Borkenschicht ab. Nach außen hin, an der Stammoberfläche, werden diese Stellen als sogenannte Teerflecken sichtbar, auffällige braun-schwarze Nekrosen in Kombination mit abgeschiedenem Wundgummi, die sich fleckig oder auch zungenförmig vom Wurzelhals am Stamm aufwärts entwickeln. Aufgrund des unterbrochenen Saftstroms bilden stark befallene Bäume nur kleinere, gelbliche Blätter, so daß die Krone insgesamt einen schütteren Eindruck macht. Häufig zeigen diese Bäume auch einen starken Fruchtansatz (Notfruktifikation). Manche Erlen erholen sich wieder, Bäume mit den Stamm umfassenden Nekrosen sterben jedoch rasch ab.

Eine bestimmte *Phytophthora*-Art, die sogenannte 'Erlen-Phytophthora', wird mit der überwiegenden Mehrheit der Fälle in Verbindung gebracht und zeigt sich als hoch-pathogen gegenüber Erlen. Bei den Erregern handelt es sich um Hybriden zwischen *Phytophthora cambivora* und einer mit *Phytophthora fragariae* nahe verwandten Art. Von beiden Pilzarten wird angenommen, daß sie in Europa ursprünglich nicht heimisch waren und vermutlich durch den Handel mit Pflanzen und pflanzlichen Produkten eingeschleppt wurden. Es ist daher wahrscheinlich, daß die 'Erlen-Phytophthora'-Hybriden ihre Entstehung dem Einfluß des Menschen verdanken.

Inzwischen ist bekannt, daß es sich bei den Erregern um eine Gruppe von Hybriden handelt, so daß manchmal auch von 'Erlen-Phytophthoras' im Plural gesprochen wird. Diese Gruppe umfaßt den weitverbreiteten 'Standard-Typ', sowie eine Anzahl von 'Varianten', von denen einige anscheinend eine nur begrenzte Verbreitung aufweisen. Die Varianten sind genetisch gesehen möglicherweise Abbauprodukte des Standard-Typs, eine andere Vermutung ist, daß sie durch Rückkreuzung oder auch durch weitergehende Hybridisierung entstanden sind. Manche der Varianten erwiesen sich als weniger pathogen als der Standard-Typ.

Die Phytophthora-Erkrankung von Erlen ist mittlerweile aus elf Ländern Europas berichtet worden: Österreich, Belgien, Frankreich, Deutschland, Ungarn, Irland, Italien, Litauen, den

Niederlanden, Schweden und Großbritannien. In einigen Gegenden sind sehr hohe Verluste zu verzeichnen, während die Krankheit in anderen Regionen nur geringe Auswirkungen hatte. In manchen Untersuchungsgebieten entlang von Fließgewässern nahm die Absterberate der Erlen schnell und unaufhaltsam zu, so z.B. im Süden Englands und im Nordosten Frankreichs, wohingegen sich der Bestand anderer Regionen, wie z.B. in Oberösterreich, bemerkenswert von dem Befall erholt hat. Das Feuchtgebiet 'Die Wieden' in den Niederlanden beherbergt eine pathogene Variante des Pilzes, die anscheinend keinen erkennbaren Schaden verursacht. Noch ist wenig bekannt, welchen Einfluß die Lebenskraft der Wirtsbäume auf das Ausmaß der Erkrankung hat, ein solcher Zusammenhang mag aber mit ein Grund sein für das plötzliche Auftreten der Erkrankung an zwei Bächen im südlichen Teil der Niederlande im Anschluß an ein künstliches Anheben des Wasserspiegels.

Möglichkeiten, eine Ausbreitung des Erregers einzudämmen

Es ist bereits weitläufig bekannt, welche wichtige Rolle Fließgewässer verschiedenster Größe für die Ausbreitung der Erkrankung spielen. Sobald der Erreger in ein Fluß-System gelangt, ist seine Ausbreitung über begeißelte Sporen, die mit der Strömung flußabwärts getragen werden, unvermeidlich. Erlen im Uferbereich der Wasserläufe können dann neu infiziert werden.
Es gilt als gesichert, daß Phytophthora-Erreger mit infiziertem Pflanzmaterial aus Baumschulen in zuvor unbefallene neue Gebiete eingeschleppt worden sind. Um eine solche Verschleppung über weite Strecken in der Zukunft auszuschließen, empfehlen die Mitglieder der Internationalen EU-Arbeitsgruppe, jenem Pflanzmaterial, das in noch unbefallene Regionen Europas und der restlichen Nordhalbkugel eingeführt werden soll, strenge pflanzenschutzliche Kontrollmaßnahmen aufzuerlegen. Weiterhin wird vorgeschlagen ein Zertifizierungssystem für Erlenpflanzen aus Baumschulen zu entwickeln, sobald die Methoden zur molekularen Erkennung der Erreger ausgereift sind. Bis dahin sollte verantwortungsvolles Handeln von den Baumschulen praktiziert werden. Dabei sollte gewährleistet sein, daß kein Anschluß des Bewässerungssystems der Baumschule an Fließgewässer besteht, das Erlen-Pflanzmaterial während der Wachstumsperiode regelmäßig auf Symptome kontrolliert wird und routinemäßige Desinfektionen des Baumschulengeländes zwischen den Pflanzungen durchgeführt werden.

Managementkonzept für die Krankheit

Untersuchungen, inwieweit sich infizierte Erlen durch Rückschnitt ('Auf-den-Stock-setzen') behandeln lassen, befinden sich noch in einem frühen Stadium, da erst wenig darüber bekannt ist, wie anfällig die Stockausschläge gegenüber der Krankheit sein werden. Eine direkte Bekämpfung des Erregers mit Hilfe von Fungiziden ist noch nicht in Betracht gezogen worden und wird sich wohl in der näheren Zukunft auch nicht als vielversprechend zeigen können. Bis dato haben Untersuchungen zur Wirtsresistenz eher enttäuschende Ergebnisse gebracht, was die Vermutung nahelegt, daß Resistenzgene im Wirt wahrscheinlich nur selten auftreten. Ein langangelegtes Untersuchungsprogramm wird sein. Allerdings dürfen für eine solche Studie die zur Klärung erforderlich Wandlungsfähigkeit und vielfältige Ausprägung, sowie Stabilitätseigenschaften des Erregers nicht außer acht gelassen werden.

Forschungsbedarf für die Zukunft

Die gemeinsame Forschung einer internationalen Arbeitsgruppe im Rahmen eines EU-Projektes kann nur in dem Maße geschehen, wie es die Finanzierung der einzelnen Partnerorganisationen zuläßt. Dabei haben sich die teilnehmenden Partnerorganisationen auf die folgenden Forschungsschwerpunkte für die Zukunft geeinigt:

- Entwicklung von molekularbiologischen Untersuchungsmethoden zur Identifizierung der Erlen-Phytophthoras in Boden, Wasser und Wirtsgewebe.
- Aufklärung des Infektionsvorgangs, einschließlich der Fragen zum Überleben des Erregers, seiner Fortpflanzung und Ausbreitung.
- Verständnis der den Wirtsbefall beeinflussenden Faktoren, mit besonderer Berücksichtigung dessen, welche Auswirkungen Umweltfaktoren auf die Fähigkeit des Wirtes haben, dem Erreger zu widerstehen.
- Erweiterung des Kenntnisstands zur Wandlungsfähigkeit und vielfältigen Ausprägung, sowie der Stabilität der Genotypen für Erlen-Phytophthora, die in Europa gefunden wurden.
- Einrichtung und Unterhalt von Versuchsflächen für die Erstellung von Modellen und Vorhersagen zum Krankheitsverlauf.
- Resistenzuntersuchungen von Wirtspflanzen gegenüber dem Erreger, mit besonderem Augenmaß auf solche Bäume, die in Gebieten mit hoher Erlensterberate befallsfrei bleiben.
- Untersuchungen dazu, welche Rolle Baumschulen bei der Ausbreitung der Erkrankung spielen.
- Entwicklung von Pflanzenschutzkonzepten gegen Erlensterben für Baumschulen.

Crynodeb

Statws cyfredol y cynhaliwr a'r clefyd

Mae gwerni'n chwarae rhan hanfodol bwysig yn Ewrop. Mae ganddynt amrywiaeth o nodweddion sydd yn eu galluogi i ymsefydlu fel arloeswyr ac sydd hefyd yn caniatáu iddynt, o leiaf yn achos y wernen gyffredin *Alnus glutinosa*, chwarae rhan sylweddol mewn coedwigoedd brig. Mae *A. glutinosa* hefyd yn gwneud cyfraniad pwysig i ecoleg a sefydlogrwydd glannau afonydd. Mae pob un o'r pedair rhywogaeth o'r wernen yn Ewrop yn bwysig wrth sefydlu coetir ar safleoedd anodd.

Ar wahân i glefyd Phytophthora, mae gwerni Ewropeaidd i raddau helaeth yn rhydd o broblemau plâu a chlefydon pwysig. Gall pryfed penodol sydd yn tyrio i'r boncyff achosi gwywo amlwg ac yn aml ymhlygwyd sychder fel achos difrod. O bryd i'w gilydd cafwyd gwywiadau heb achos amlwg. Ond mae dadansoddiad beirniadol o'r llenyddiaeth wedi gwaredu'r syniad i raddau helaeth bod clefyd Phytophthora yn bresennol ymhell cyn iddo gael ei gydnabod ym Mhrydain ym 1993. Ymddengys bellach yn annhebygol iddo fod mewn bodolaeth am lawer mwy na 30 mlynedd. Y symptom allweddol yw anaf madreddog yn rhisgl mewnol y boncyff ac yn aml nodweddir yr anaf hwn yn allanol gan archwys tarllyd neu rydlyd. Mewn coed ag effeithiau difrifol arnynt, mae'r deiliant yn fach, prin ac yn aml yn felynllyd. Mae ffrwytho trwm yn gyffredin. Fel arfer bydd coed o'r math yn marw'n weddol gyflym ond mae'n bosibl y bydd rhai yn gwella.

Mae rhywogaeth arbennig *Phytophthora* – a adweinir fel 'Phytophthora y wernen' – yn gysylltiedig â'r mwyafrif llethol o achosion a dangoswyd bod honno'n bathogenaidd iawn i werni. Mae'n tarddi o groesiad – gyda *P. cambivora* a ffwng yn agos i *P. fragariae* yn 'rhieni'. Ni chredir bod ffyngau yr un na'r llall yn frodorol i Ewrop, ac maent yn debygol o gael eu cludo i mewn yn ddamweiniol gan y fasnach mewn planhigion neu gynhyrchion planhigion. Felly mae gweithgareddau dynol ryw yn debygol o fod yn gyfrifol am darddiad Phytophthora y wernen. Gwyddys ei bod yn rhan o haid o glefydau ac felly defnyddir y term ' Clefydau Phytophthora y Wernen' weithiau. Elfennau'r haid yw'r 'math safonol' â dosbarthiad eang a nifer o 'amrywiolion' y mae'n ymddangos bod i rai ohonynt ddosbarthiad cyfyngedig. Gall yr amrywiolion fod yn gynhyrchion dirywiad genetig y math safonol neu gallant fod yn gynhyrchion ôl-croesiadau neu ddigwyddiadau croesiadau pellach. Mae rhai o'r amrywiolion yn llai pathogenaidd na'r math safonol.

Cofnodwyd y clefyd bellach mewn un-ar-ddeg o wledydd: Yr Almaen, Awstria, Yr Eidal, Ffrainc, Gwlad Belg, Hwngari, Yr Iseldiroedd, Iwerddon, Lithiwania, Sweden a Phrydain. Cafwyd colledion uchel iawn mewn nifer o leoedd tra bu effaith y clefyd mewn lleoedd eraill yn fach. Mae nifer o leiniau arolwg ar lannau afonydd yn dangos cynnydd cyflym a diarbed mewn marwolaethau (er enghraifft yn Ne Prydain a Gogledd Dwyrain Ffrainc) tra bod eraill (e.e. Uwch-Awstria) yn dangos lefel uchel o adferiad o'r clefyd. Mewn man penodol, sef gwlyptir naturiol Die Wieden yn yr Iseldiroedd, mae amrywiolyn pathogenig o'r ffwng yn bresennol heb achosi unrhyw ddifrod gweladwy. Ychydig a wyddys am ddylanwad hoenusrwydd y cynhaliwr ar ddatblygiad y clefyd, ond gall hyn fod yn ffactor yn ymddangosiad sydyn y clefyd mewn dwy nant yn rhan ddeheuol yr Iseldiroedd ar ôl i lefelau'r dŵr gael eu codi'n artiffisial.

Posibiliadau atal lledaeniad y pathogen

Mae pwysigrwydd afonydd a nentydd wrth ledaenu'r pathogen yn hysbys iawn, ac unwaith y bydd y pathogen yn bresennol mewn system afon, mae dosbarthiad i lawr y llif yn anochel. Mae

tystiolaeth gref bod gwerni o fethrinfeydd wedi chwarae rhan bwysig wrth ledu'r pathogen i ardaloedd newydd. Er mwyn cadw lledaeniad hirbell ychwanegol o'r pathogen trwy'r dull hwn, mae Partneriaid yn y Gweithredu ar y Cyd yn cymeradwyo gosod rheolau iechyd planhigion llym ar blanhigion yn dod i mewn i wledydd yn Ewrop a mannau eraill yn Hemisffer y Gogledd sydd yn rhydd o'r clefyd. Hefyd maent yn argymell datblygu cynllun ardystio ar gyfer planhigion gwerni o'r feithrinfa unwaith y bydd technegau darganfod molecwlar wedi'u datblygu. Hyd hynny, cymeradwyir datblygiad Côd Arferion Da. Ymhlith pethau eraill byddai hyn yn golygu gwaharddiad ar ddyfrhau o'r feithrinfa o afonydd, archwiliad iechyd y planhigion gwerni yn ystod y tymor tyfiant a diheintiad rheolaidd o'r feithrinfa rhwng cnydau.

Dewisiadau am reoli'r clefyd

Mae astudiaeth o brysgoedio'r coed afiach yn eu dyddiau cynnar, ac ychydig sydd yn hysbys am y lefelau o glefyd y gellir eu disgwyl yn y tyfiant newydd. Ni arbrofwyd â thriniaethau cemegol hyd yn hyn, ond maent yn annhebygol o gynnig llawer o bosibiliadau am gael eu defnyddio. Hyd yn hyn, bu canlyniadau astudiaethau o wrthsafiad y cynhaliwr yn siomedig, sydd yn awgrymu y gall genau gwrthsafiad fod yn brin a bydd angen rhaglen sgrinio gwrthsafiad hir-gyfnod. Am waith o'r math, bydd rhaid ystyried amrywiaeth ac ansefydlogrwydd yn y pathogen achosol.

Anghenion ymchwil i 'r dyfodol

O dan y cynllun Gweithrediad ar y Cyd, gall ymchwil ddatblygu ond i'r graddau y bydd ariannu gan y cyrff partneriaethol yn ei chaniatáu. Mae'r Partneriaid wedi cydnabod y blaenoriaethau allweddol canlynol ar gyfer gwaith i'r dyfodol:

- Datblygu taclau molecwlar am adnabod pathogenau Phytophthora y wernen mewn pridd, dŵr a meinwe'r cynhaliwr.
- Deall y broses heintio, gan gynnwys goroesiad y pathogen a lledaeniad yr eginyn.
- Deall ffactorau sydd yn effeithio ar ymlediad i'r cynhaliwr, gyda chyfeiriad arbennig i effeithiau ffactorau amgylcheddol ar allu'r cynhaliwr i wrthsefyll effeithiau'r pathogen.
- Estyn gwybodaeth o amrywioldeb a sefydlogrwydd genoteipiau clefydau Phytophthora y Wernen ar geir ledled Ewrop.
- Sefydlu a chynnal lleiniau monitro i ganiatáu modeli a darogan datblygiad y clefyd.
- Sgrinio am wrthsafiad i'r pathogen, gan gymryd i ystyriaeth arbennig coed sydd yn rhydd o'r clefyd mewn ardaloedd o farwolaeth uchel
- Archwilio rhan meithrinfeydd wrth ledu'r clefyd.
- Datblygu strategaethau rheoli'r clefyd mewn meithrinfeydd.

In 1993 a lethal Phytophthora disease of common alder *Alnus glutinosa* was identified at several places in the UK by R. G. Strouts of the Forestry Commission's Disease Diagnostic and Advisory Service (Gibbs *et al.*, 1994). The key symptoms were typical of Phytophthora root and collar diseases of other broadleaved trees. The leaves were abnormally small, yellow and sparse; dead roots were present and strips of dead bark, extending up from collar level, occurred on the stems. Stem lesions were often marked externally by the presence of tarry or rust-coloured exudations. Further investigations later in 1993 and in 1994 revealed that the disease was widespread in southern Britain and that it was most commonly found in trees growing on or near the banks of rivers or other areas of water (Figure 1.1). However, it was also found in orchard shelterbelts and in newly planted woodland. Most cases involved *A. glutinosa* but the disease was also found in two other European species – grey alder (*A. incana*) and Italian alder (*A. cordata*). Surveys of river-bank alder populations showed that in some parts of the country, thousands of trees were dying from the disease (Gibbs, 1995).

It was soon established that the disease was caused by an unusual new fungus that had some similarities to *Phytophthora cambivora*, well-known as a cause of root and stem disease on a variety of broadleaved trees but not recorded from alder. This new fungus was described as being distinct from *P. cambivora* in its colony morphology in culture, in being homothallic rather than outcrossing, in having a high frequency of zygotic abortion, and in exhibiting a lower temperature optimum for growth and a lower growth temperature maximum (Brasier *et al.*, 1995). The work was conducted principally on isolates of the fungus from the UK. However, an isolate from a diseased *A. glutinosa* in the Netherlands, provided by the Netherlands Plant Protection Service, proved to resemble UK isolates in its homothallism and the morphology and development of its reproductive structures,

Figure 1.1 Alders killed by the disease along the River Lugg in Herefordshire (J. N. Gibbs).

although it differed in colony appearance and in its upper temperature limit for growth. Brasier *et al.* (1995) suggested that the fungus might be a species hybrid involving *P. cambivora* as a parent. Pending a full description, it was named the 'alder Phytophthora'.

Because of the possible quarantine implications, J. N. Gibbs of the UK Forestry Commission made a presentation on the disease to the EU Standing Committee on Plant Health in April 1995, during which details on symptomatology and isolation of the pathogen were disseminated. Later that year, a report was published apparently describing the same disease from two stands of *A. glutinosa* in the southern part of the Luneberger Heide in Germany (Hartmann, 1995).

In January 1996, an *ad hoc* working group on the disease was convened in Brussels under the auspices of the Standing Committee on Plant Health to determine if there was a case for introducing plant health controls. At that meeting, information on the status of the disease was discussed and it was concluded that while more information was clearly required on disease distribution, disease biology and disease control, there were insufficient grounds for recommending that plant health measures should be instituted. Key to this decision was the revelation that the unusual form of *P. cambivora* implicated in the disease had recently been isolated quite readily from the soil of the Dutch fenland nature reserve Die Wieden in a situation in which it was not associated with any obvious disease symptoms on alder (C. van Dijk, unpublished). This suggested that the fungus might be a long-term component of some natural ecosystems and that consequently plant health regulations on the movement of plants and plant products would be of little benefit in minimising its impact.

To progress matters further, an informal meeting was held in the UK in June 1996 involving scientists from eight European

countries and it was as a direct result of this meeting that a proposal for a Concerted Action on the disease was developed. The proposal was approved by the EU Authorities and came into effect on 1 March 1998. The overall objectives were to draw together information on the current and potential impact of the recently identified Phytophthora disease of alder in order to:

1. Determine if the spread of the disease within Europe can be limited.

2. Make recommendations on disease management and control.

3. Identify research requirements.

This publication is a key output from the Concerted Action. In Chapter 2, by Hugues Claessens, the nature and status of the host population in Europe is described. The common or black alder (*A. glutinosa*) is the most important species. It occurs widely in Europe, dominates whole ecosystems and, over the years, its wood has been of considerable significance to the economy of many areas. The grey alder *(A. incana)* is also widely distributed and has often been planted outside its natural range. The Italian alder *(A. cordata)* has a very limited natural distribution but, with its handsome glossy leaves, has found an important place in many tree planting schemes during the last 50 years. Finally, there is the green alder *(A. viridis)*, a shrubby species, which plays a useful role in avalanche and erosion control in the Alps and elsewhere.

In Chapter 3, Thomas Cech and Steven Hendry present information on dieback and decline diseases of alder as they have been reported across Europe during the last two centuries, and provide an analysis of putative cause. They consider whether Phytophthora disease might have been present in Europe well before it was formally recognised and described.

Chapter 4 by Jean-Claude Streito is concerned with the identification and distribution of the Phytophthora disease of alder. A key part of the work of the Concerted Action was to exchange information on suitable techniques for the isolation of *Phytophthora* species from alder and the results of this work are described. Alder Phytophthoras, as described by Brasier *et al.* (1995), were by far the most common Phytophthoras to be isolated from diseased trees but other species of *Phytophthora* were sometimes recorded and these occurrences are summarised. Records of the pathogen from the various countries of Europe are presented and the chapter ends with a reassessment of the key symptoms of the disease across the continent.

Chapter 5 by Clive Brasier deals with the nature of the causal organism and its hybrid origin. It provides information on the characteristics of the 'standard' and the 'variant' types of alder Phytophthora and discusses their relationships to each other. Data are provided on the pathogenicity of the alder Phytophthoras to various woody hosts and on the capacity of the fungus for saprotrophic survival.

In Chapter 6, John Gibbs, Thomas Cech, Thomas Jung and Jean-Claude Streito present information on the dissemination of the pathogen by various means, in particular via watercourses and on nursery plants. This chapter also reviews data on the impact and severity of the disease. The longest sequence of data on disease development in permanent plots comes from the UK and this is presented together with shorter data sequences from Austria and France. Suitable evaluation of disease survey data can provide circumstantial evidence for the importance of environmental factors on disease development, and information is presented on this subject.

Chapter 7 by David Lonsdale outlines current knowledge on inoculum sources, processes of infection and host invasion. Emphasis is laid on the important gaps in our

knowledge that still remain but the potential agents of infection (oospores and zoospores) and the infection court are considered.

In Chapter 8, John Gibbs considers some options for disease management and control. Data are presented on the possible role of the coppicing of affected trees to promote regeneration and on the disease susceptibility of different provenances of *A. glutinosa*.

Finally, Chapter 9 by Cees von Dijk and John Gibbs considers the accumulated information in relation to the objectives of the Concerted Action. There is an evaluation of whether the spread of the disease in Europe can be limited, proposals are made for disease management and control, and research requirements are identified.

Much information was exchanged in the Workshops that formed an integral part of the work of the Concerted Action (CA). Where not published elsewhere, such information is cited as 'Records of the CA'.

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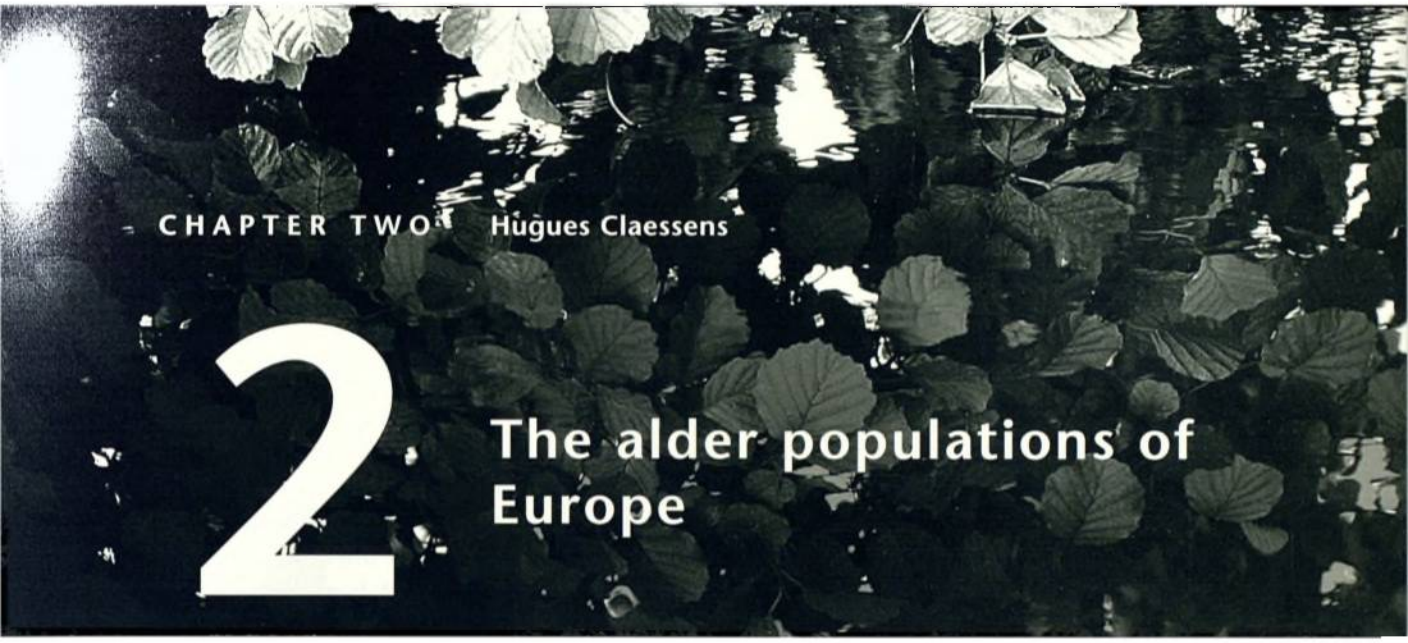


CHAPTER TWO Hugues Claessens

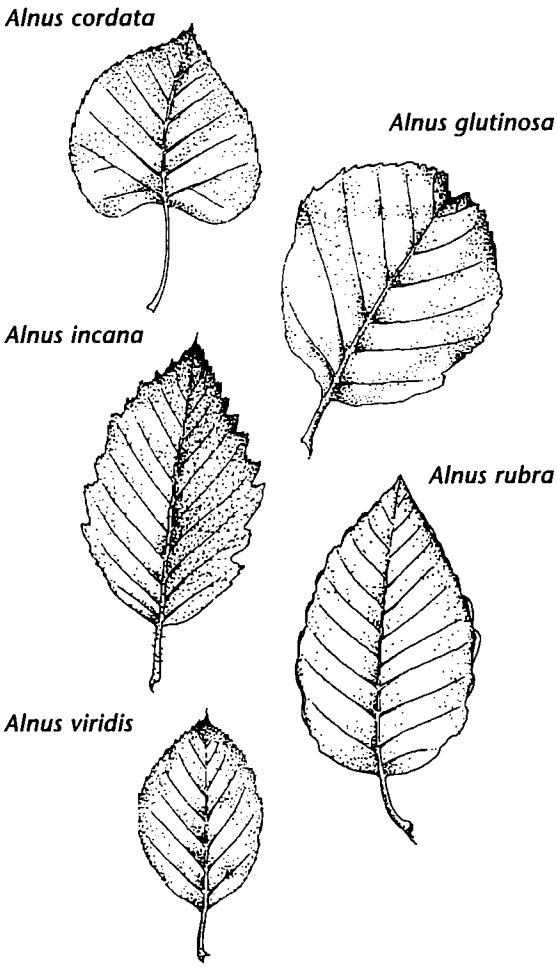
2 The alder populations of Europe

Introduction

The genus *Alnus* is distributed across the Northern Hemisphere. There are considered to be about 35 species, although there is much disagreement over species delineation in some cases. *Alnus* is principally a pioneer genus with the associated characteristics of wind pollination, seed dispersal by wind and water, rapid colonisation of bare ground and open herbaceous vegetation, fast initial growth, intolerance of shade and a relatively short life span. Many alder species are tolerant of high groundwater levels and periodic flooding, and some are tolerant of salinity. A special feature of alders is their persistent symbiotic association with *Frankia*, an actinomycete which is able to fix atmospheric nitrogen in specialised root nodules. This nitrogen, fixed at rates of 60–400 kg h^{-1} yr^{-1}, is available both to the host tree and to the environment.

There are four alder species native to Europe: the common or black alder (*A. glutinosa*), the grey alder (*A. incana*), the Italian alder (*A. cordata*) and the green alder (*A. viridis*). In addition, the North American red alder (*A. rubra*) has been quite extensively planted in some countries. The recognition that a lethal Phytophthora disease occurs on alder in a number of European countries made it important for the nature and significance of the host population to be considered. This chapter reviews information on the characteristics of each of the European species and on their ecological and economic importance. *The forestry compendium* (CAB International, 2000) or other general texts should be consulted for details of the taxonomic features of each species, as these are not considered here. However, the leaves of the four European species and *A. rubra* are shown in Figure 2.1. The account of black alder is based largely on Claessens (1999) and Claessens and Thibault (1994). The accounts of grey alder, Italian alder and green alder draw on the information in *The forestry compendium* and on articles in the *Enzyklopädie der Holzgewächse*, such as Grossoni (1997).

Figure 2.1 Leaves of the four alder species commonly grown in Europe and the North American red alder (from White and Gibbs, 2000).



Black or common alder (*A. glutinosa*)

Black alder is the most abundant and widely distributed of the alder species of Europe. It is particularly well adapted to wet sites and plays a vital ecological role in relation to the protection of soil and water. In addition it can produce a high quality wood of considerable market value. At maturity it can reach 25–30 m in height with a stem diameter at breast height (dbh) of over 1 m.

Distribution and habitat

Black alder is a very common tree of riverbanks and damp forests across most of Europe (Figure 2.2). In the dry continental terrain of the east and in Mediterranean regions it only occurs along streams. In terms of altitude it is present to 1200 m in the south (Pyrénées), 1800 m in the Alps and 1500 m in eastern Europe. In the north, it does not occur in zones where the frost period lasts for more than 6 months (MacVean, 1953).

Glavac (1972) identifies two major regions where large stands of black alder occur. One of these is in the plains of north-central Europe (northern Germany, Poland, Bielorussia, Lithuania) where black alder stands represent 5% of the forest area. Some of these, such as the Spreewald which covers around 2000 ha in northeast Germany, are famous. The second major region is located in the Danube plains (Austria, Slovenia, Croatia) where black alder forms large pure stands and is sometimes called 'valley spruce'. Important local stands can be found in many other countries, especially along waterways, and the species comprises about 1% of total forest cover (Turok *et al.*, 1996).

Man has had a very important influence on the abundance of black alder. Natural wetlands have been drained or covered with sediment during the progressive deforestation in Europe. Loamy plateau forests have been converted to agriculture after drainage. River sites have been changed through the construction of navigation and flood control systems. In addition, forest management systems, particularly in western Europe, have favoured the reduction of alder and its replacement by more commercial species.

However, in contrast to losses elsewhere, black alder has been maintained and encouraged on river banks for its ecological benefits in relation to river ecosystems and bank stabilisation. At least in western Europe, these stands now represent the most important component of the black alder population and provide a witness to the former existence of

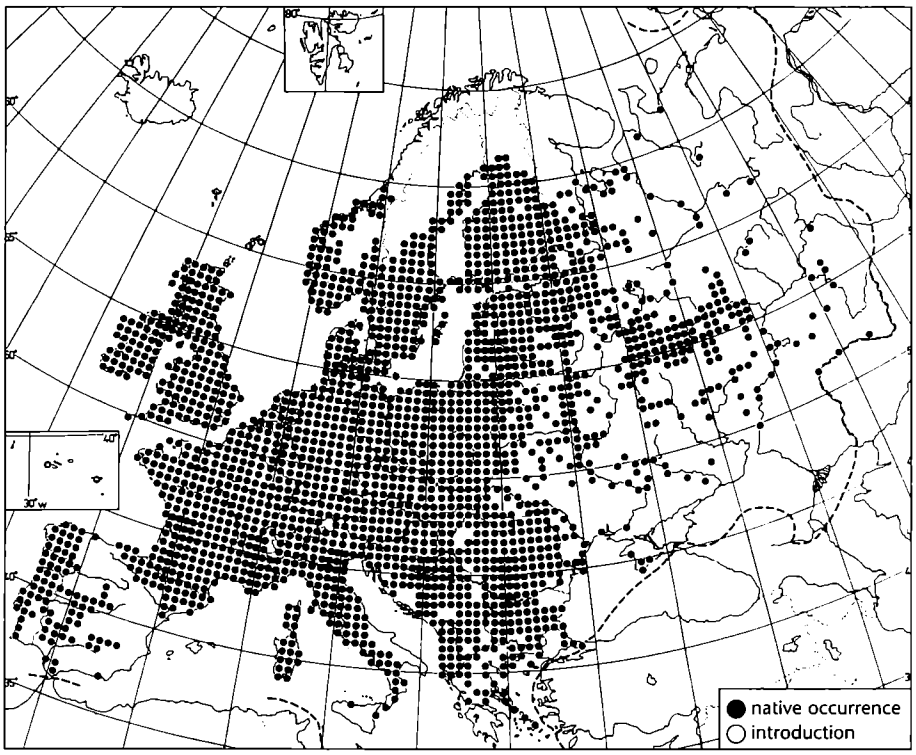


Figure 2.2 Distribution of *Alnus glutinosa* in Europe (from Jalas and Suominen, 1976). It is also present in Northern Turkey, Caucasus and in some valleys in Maghreb.

riparian forests of this species. In future the requirement for alder may increase in relation to the development of EU policies on the need to preserve and enhance groundwater supplies and wetland areas.

Biology

The eco-physiology of growth in black alder has been studied by Eschenbach (1996). The species combines the pioneer qualities of the genus (outlined in the Introduction) with a special relationship to wet sites. Three major site types can be identified:

- Marsh sites with a waterlogged subsoil throughout the year.
- River sites in which the soil in the rooting zone is well drained during the growing season.
- Plateaux sites on deep loamy soils with temporary watertable and high soil moisture content.

Adaptation to wet sites

Attributes showing adaptation to wet sites include the presence of abundant cork cells in the seeds which enable them to float on the surface of the water for up to a year without loss of germination capacity. Germination also requires moist conditions and cannot occur if the relative humidity of the air falls below 50%, a level that must also be maintained during the first month after germination for the seedlings to survive.

The root system of alder consists of several major horizontal roots which, together with their associated feeder roots and root nodules, colonise the aerated surface layers of the soil. In conjunction with these, a system of strong vertical roots is produced which can go deep into asphyxiated soil (McVean, 1956). Kostler *et al.* (1968) observed roots which were nearly 4 m deep. Under anaerobic conditions, the oxygen supply comes from the aerial parts of the tree via well-developed aerenchyma cells

linked to large lenticels on the stem. These cells can also enable toxic gases to be evacuated. In addition the metabolism of alder is adapted to reduce the production of toxic substances during anaerobiosis (Crawford, 1992).

Loss of function in the rooting system due to prolonged flooding or other damage (as caused, for example, by a pathogen like *Phytophthora*) can also induce the development of adventitious roots (Gill, 1970, 1975). These are non-geotropic roots growing from lenticels and appearing at the soil surface or just below the surface of any water (Figure 2.3). The large lacunae between the cells enable efficient passage of oxygen. These roots generally die when the floods disperse. The structure of the lenticels and the development of adventitious roots during flooding is of considerable interest to a possible understanding of the infection mechanism for the Phytophthora disease of alder (see Lonsdale, Chapter 7).

Figure 2.3 Adventitious roots on the lower stem of a young tree of *Alnus glutinosa*. These can be produced following prolonged flooding of the root system or in response to death of the bark; here they have been produced as a result of Phytophthora disease (H. Claessens).

Growth on dry sites

The capacity to produce a large and deeply penetrating root system enables the black alder to exploit a large volume of soil and this can allow successful growth on relatively dry sites. However, the leaves cannot control their transpiration (Herbst *et al.*, 1999), and the normal response to drought conditions is for the tree to drop its leaves.

Genetic variation

With such a large area of distribution, including Mediterranean, central European and Atlantic biogeographic zones, it is not surprising that geographically-based ecotypes occur. Three ecotypes that differ from the 'typical' alder have been identified:

- a northern-Atlantic ecotype (Netherlands, Northern Belgium);
- a Scandinavian ecotype adapted to long days and low temperatures;
- a southern Europe ecotype, adapted to growth conditions of Danube plain.

This local adaptation means that Scandinavian alders grow as well in their native habitats as German alders do in theirs (Glavac, 1972). In the Walloon region in Belgium, Claessens (1999) has shown that the productivity of stands correlates well with the length of the growing season and temperature.

Ecological and economic importance

Black alder has a wide ecological role and a beneficial effect on water quality and flood control on many sites. It has a significant part

to play in soil improvement and afforestation schemes and also produces a wood that can have appreciable commercial value.

Biological value

The position of alder forests, at a transition between mesic soil and water ecosystems, means that they comprise an important range of habitats, most of which are protected by European or national directives. Also, as a pioneer species, alder plays a key part in forest dynamics and forest succession.

Alders can make a valuable contribution to biodiversity. For example the seed, which is liberated progressively during the winter, provides food for many birds. Investigations in the Balowieza Forest in Poland have shown that alder carrs have a diversity of bird fauna three times greater than that of mixed stands and coniferous stands (Tomialojac *et al.*, 1984). Black alder also has many mycorrhizal associates, some of which are only found on alder. Where alders are growing along waterways, the shade cast by the trees may be important in regulating the amount of 'in-stream' vegetation, insects falling from the leaves may be an important source of food for fish and the roots of the trees can provide safe fish spawning grounds. For a review of this subject see Dussart (1999).

Effects on water quality and flood control

Riparian woodlands of black alder form a buffer zone along many streams and play a vital part in the filtration and purification of water. Several authors provide evidence for very efficient denitrification and phosphate 'trapping' in such wet woodlands (Pinay and Labroue, 1986; Peterjohn and Correl, 1984). At times of serious flooding, alluvial forests can accommodate expansion of rivers and reduce the impact of the floods on human activities. At the same time, floods in the forest can contribute to a replenishment of good quality groundwater (Schnitzler and Carbiener, 1993).

Black alder as an improvement species

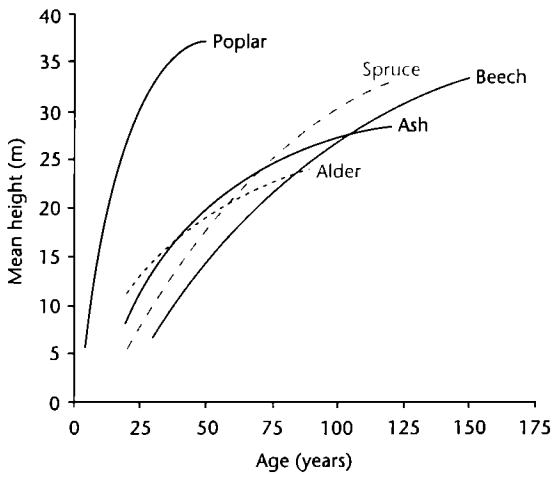
The ability of black alder to fix atmospheric nitrogen has resulted in its being used for the improvement of poor soils, including some on reclamation sites which are really quite dry. There is an added benefit in that the strong rooting action of black alder improves the porosity of the soil, which becomes more aerated and favourable for plant growth. On some humid sites, there can be the additional benefit of high water consumption, in effect biological drainage.

Black alder can form a useful 'nurse' species in afforestation schemes. Where it has been interplanted with a more commercial species, such as walnut *(Juglans* spp.), poplar *(Populus* spp.) or Douglas fir *(Pseudotsuga menziesii),* various authors have shown an increase in the growth of the primary species from 20 to 100% according to the experimental conditions (Schlezinger and Williams, 1984). In Wallonia, trials of mixed black alder and Norway spruce *(Picea abies)* have been established on humid and poorly structured oligotrophic soils to increase the soil nutrient status and porosity to the benefit of both soil quality and the productivity of the spruce. In several countries (the Netherlands and the UK for example) black alder has been used frequently in orchard shelterbelts where it enhances the productivity of the adjacent fruit trees. Recently, however, this role has increasingly been taken over by *A. incana* and *A. cordata.*

Silviculture and productivity

Growth studies and published yield tables (e.g. Schober, 1975 for Germany) emphasise the particular growth pattern of black alder. Figure 2.4 compares height growth of various forest species and shows that black alder is characterised by rapid early growth which declines quickly. Maximum annual wood volume increment (up to 15 $m^3\ ha^{-1}\ yr^{-1}$) is greater than that of other fast-growing

Figure 2.4 Growth rate of different forest trees from yield tables of Schober (1975) in Germany.



broadleaved species and is reached earlier (at around 20 years old). These growth characteristics have an important influence on the possibilities and techniques for alder cultivation. Early thinning and short rotations can make the best use of the fast early growth of black alder (Fourbisseur, 2000). Because of its rapid early growth, there has been some interest in black alder for short rotation coppice to produce biomass. However, at present, it is not a favoured species for this purpose.

Wood products and timber market

In the past black alder has had many specific uses. Its durability under water has meant that it has been used for such items as sluices, pumps and troughs. Boat bottoms and punts were also made of alder. In the Middle Ages alder was used for the foundations of the houses in Amsterdam. The ease with which it can be carved or turned also resulted in a number of specific uses: for example, for the production of clog soles and break-pads on mine trolleys. It can be made into a fine even-grained charcoal, which is excellent for use in the manufacture of top quality gunpowder. Indeed alder woods were often planted around gunpowder factories to ensure a ready supply of suitable wood (White and Gibbs, 2000). Today one of the best uses of alder wood is for veneer, especially for manufacturing plywood. This use requires large defect-free trunks. There is also a market for solid wood in furniture production. Although black alder is widespread in the forests of Europe, timber markets are only well developed in regions where the tree covers large areas. Stumpage prices can vary from 400 to 800 EURO for the best quality timber consisting of stems without faults, 50 to 60 cm dbh.

Grey alder (*A. incana*)

The grey alder is a widely distributed tree with an important ecological role. At maturity it reaches 15–20 m in height with a stem dbh of 40 cm.

Distribution and habitat

The distribution of grey alder is shown in Figure 2.5. It is a lowland tree in the northern part of its range and a montane one to the south. In Eastern Europe its range is broadly similar to that of black alder but it differs markedly in other areas. Thus it is common in northern Scandinavia where black alder is absent and does not occur naturally to the west of the line from the River Rhone in southeast France and the River Oder in northern Germany. It has been widely planted both within its natural range and elsewhere and in some regions it can be difficult to distinguish wild populations from planted ones. Grey alder is mostly found on good soils but will also grow on poor dry sites. It commonly occurs along shorelines. Unlike black alder, it suckers readily and this leads to the development of quite large stands of the same genotype.

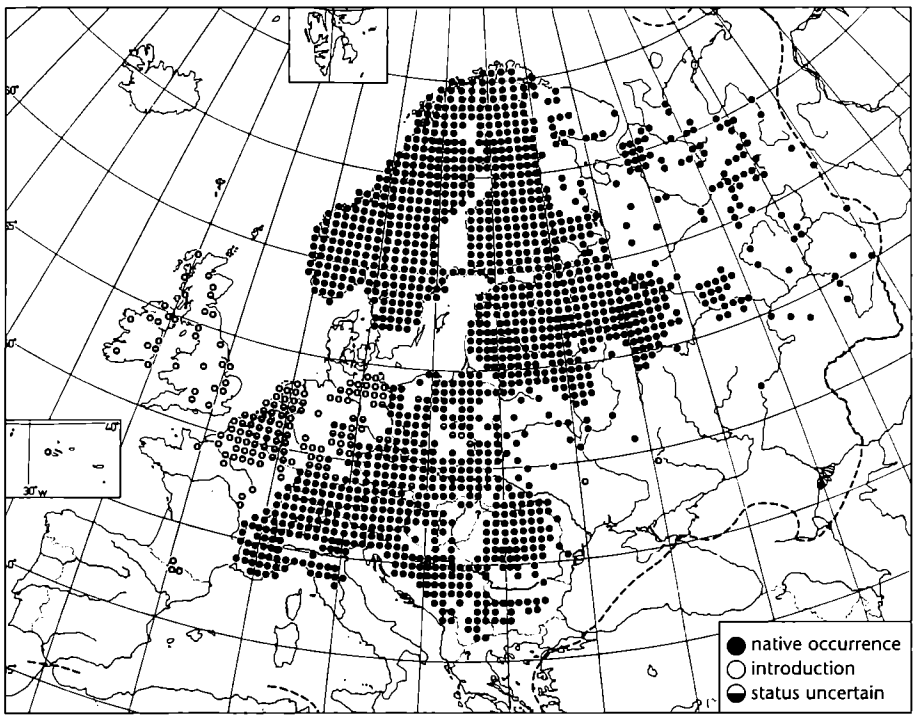


Figure 2.5
Distribution of *Alnus incana* in Europe (from Jalas and Suominen, 1976).

Biology

The biology of grey alder has been relatively little studied. It resembles black alder in its ecological behaviour although it is rather more tolerant of shade (Schwabe, 1985). It is less tolerant of flooding than black alder although short periods of waterlogging are not harmful. An ability to grow in northern Scandinavia indicates that it is very resistant to frost. Like black alder, it also has systems of both horizontal and vertical roots, the latter being known to penetrate 90 cm into the soil (Schwabe, 1985). Adventitious roots can be produced on the stem in response to flooding.

Genetic variation

Several subspecies have been recognised but there has been little work on the existence of ecologically distinct entities within the species. Natural hybrids with *A. glutinosa* occur where the species coincide and in the field these hybrids are hard to distinguish from grey alder.

Ecological and economic importance

In montane areas grey alder often grows on the banks of streams and plays a significant role in erosion control. Because of its ability to grow on dry soils it has been widely used as a soil improver and as a nurse species for tree establishment on difficult sites. Its frost-hardiness is also important in this respect. Grey alder has also been used in orchard shelterbelts.

Grey alder is characterised by rapid early growth and a short life span: this rarely exceeds 50 years. The wood is generally poorer than that of black alder and in countries such as Norway where both species are found, it is less favoured. However, it can be used for the production of plywood, containers, tool handles, toys and other small items.

Italian alder (*A. cordata*)

Italian alder is a species with a very limited natural range but it has been planted widely for

a variety of purposes. At maturity, it can achieve a height of 20 m and a stem dbh of 50 cm.

Distribution and habitat

Italian alder is endemic to southern Italy and Corsica (Figure 2.6). It is found in mixed woodland on sites characterised by high rainfall and relatively mild winters.

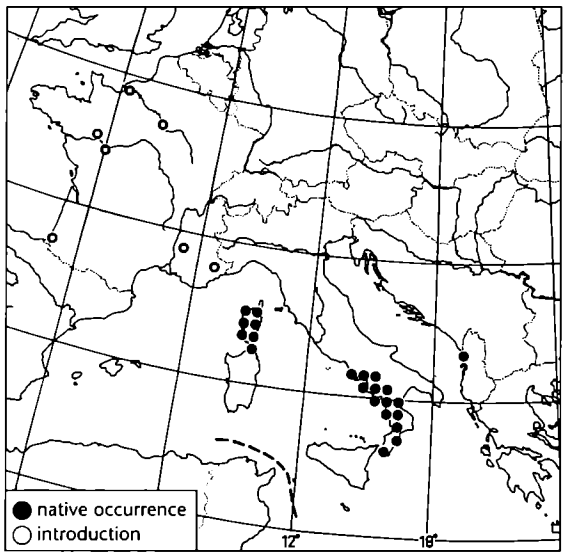


Figure 2.6 Distribution of *A. cordata* in Europe (from Jalas and Suominen,1976).

Biology

Italian alder will grow on a wide variety of soils. It has an ability to tolerate drought; unlike black alder, showing some ability to reduce transpiration rates by closing its stomata. The root system is relatively extensive; there are no strong vertical roots so it does not extend very deep into the ground.

Genetic variation

There has been no taxonomic differentiation within *A. cordata*. However, there is known to be considerable variation within and between populations and it is thought that there is abundant scope for future selection (see Grossoni, 1997). Natural hybrids occur with *A. glutinosa*.

Ecological and economic importance

In woodlands, Italian alder can be used as a nurse for other more valuable broadleaved species. However, a selection of good genotypes is important for successful plantation growth outside the natural range. In addition, its ability to grow on a wide variety of soils and to improve soil fertility means that it has been much planted in soil improvement projects. It is also used as a shelterbelt species, mainly with good results. Problems have arisen where this species has been planted on poorly drained clay soils in the Netherlands and these are under investigation (C. van Dijk and H. de Gruyter, personal communication). With its handsome glossy foliage it has found an important place in ornamental plantings. In Italy the wood has many uses such as plywood and particle board. It is also used for turning and carving.

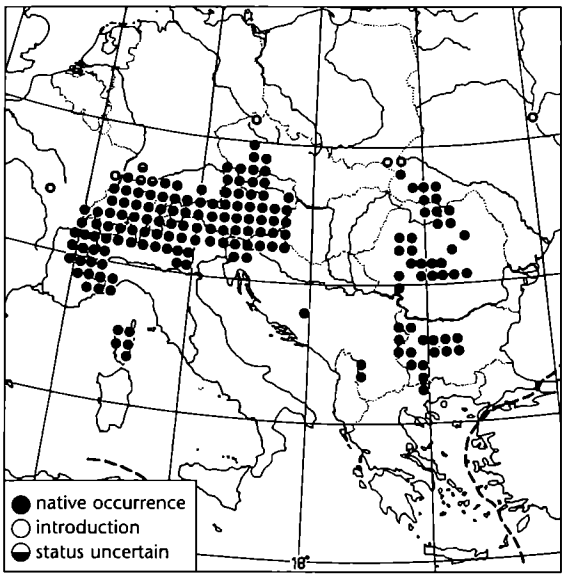
Green alder (*A. viridis*)

Green alder is a tall shrub, sometimes growing into a multi-branched tree, 4–6 m in height. Many stems often grow together to form a dense clump.

Distribution, habitat and biology

Green alder is native to the mountains of central and eastern Europe (Figure 2.7), where it can form dense thickets at altitudes between 1600 and 2200 m. It can act as a pioneer in the process of forest succession on former grassland, rapid colonisation being aided by the fact that if branches are brought in contact with the ground they will readily root.

Figure 2.7 Distribution of *A. viridis* in Europe (from Jalas and Suominen, 1976).



Genetic variation

The taxonomy of green alder is complex. Several subspecies have been recognised in Europe and it is evidently closely related to similar alders in North America (e.g. *A. crispa*).

Ecological and economic importance

In mountainous areas which experience harsh environmental conditions, green alders play an important function in preventing soil erosion and in avalanche control. In the Italian Alps, this role has been recently put in jeopardy by a serious dieback of the branches. The onset of the problem has been linked to the occurrence of very mild winters during the 1990s (G. Maresi, personal communication).

Green alder has been used successfully to nurse other tree crops on particularly difficult sites such as the china clay waste in Cornwall, UK where it has shown itself well able to tolerate exposure, soil acidity and low nutrient levels. It also has a limited use as fuel wood.

Conclusions

Both natural and planted stands of alder play a major ecological role across the European continent. This relates to their ability to stabilise riparian habitats against the effects of flood water and their ability to improve difficult sites. They often provide habitats of considerable biodiversity and under some circumstances make a significant contribution to the local economy through the production of wood.

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CHAPTER THREE

Thomas Cech and Steven Hendry

3 A review of diebacks and declines of alder (*Alnus* spp.) in Europe

Introduction

It is not the purpose of this chapter to describe all the diseases that can affect alder. Rather, an attempt has been made to collate and evaluate information on diseases in which the symptoms have some similarity to those found in the Phytophthora disease. As described by Streito (Chapter 4), the key symptom is the lesion in the inner bark of the stem; a lesion that is often marked externally by the presence of a tarry or rusty exudation. In severely affected trees, the foliage is small, sparse and often yellowish. Mortality usually follows, although recovery is known to occur, with or without the death of some branches. However, not all the symptoms are always evident. In particular, tarry spots may be absent and basal lesions difficult to find.

This review is intended to provide a useful resource for current and future studies in alder pathology. It could also indicate if any former episodes of crown damage and death represented unrecognised outbreaks of the Phytophthora disease. That this might be possible is illustrated by experience in Austria. Diseased *A. glutinosa* was investigated on the river Thaya and its tributaries near the border with Czech Republic in 1985–1987. It was concluded that the damage was due to abnormal changes of river water levels, several bark-invading and wood-decay fungi and, perhaps, frost (records from Federal Forest Research Centre 1948–1999). During the first half of the 1990s the disease reappeared and when the stands were examined in 1998, the classic symptoms of the Phytophthora disease were recognised and the presence of the fungus was confirmed (Cech and Brandstetter, 1999).

Analysis of the literature on alder diseases

For this study, numerous scientific and professional journals have been evaluated back to the first half of the 19th century. The following summary of the main findings has been compiled using a comprehensive database which contains articles from Germany, Austria, France, The Netherlands, Sweden, Switzerland, Denmark, Belgium, Czech Republic, Poland, Italy and the United Kingdom. The information is now available as an oracle database on the internet (http://fbva.forvie.ac.at/alder/cech.list).

In general, it has been found that the pathology of alders received little attention in Europe before 1900. Where accounts of damage do occur, detailed descriptions of symptoms are often lacking and only one agent of damage is usually mentioned. However, during the first half of the 20th century symptoms began to be documented in greater detail and it was recognised that in some cases a number of factors could be involved in their causation.

For the purposes of this review, an attempt has been made to classify different types of damage and disease on the basis of whether the cause is biotic, abiotic or 'complex'. A complex disease is one in which a considerable number of pathogenic agents act in concert or, more often, in sequence. Complex diseases are often characterised by poor foliage colour, a stunted growth of the whole crown lasting for several months or years and the subsequent death of some or all of the major branches: eventually the whole tree may die. In the literature, such diseases are often called 'declines and diebacks'. However, it should be noted that the second of these terms has often also been used to describe a sudden death of the branches in a situation where only a single agent of damage is involved. Various models have been devised to help in the understanding of the processes involved in complex diseases (see Manion and Lachance, 1992).

In considering the information presented below it must be recognised that attribution of cause necessarily depends upon the competence of the investigator and the research tools available. It must also be remembered that a tree only has a limited repertoire of symptoms that it can display. As a result, it is commonly the case that very similar symptoms can be produced by different combinations of damaging agents.

Biotic damage

Insects

The majority of reports of damage to stems concern the mottled willow borer (*Cryptorhynchus lapathi*). Attack by the developing larvae can occur on stems as small as 2 cm diameter, making them very vulnerable to breakage. Records of such damage come from Germany (Saxony, Bavaria, Mecklenburg/Schwerin) in 1845, 1859 and 1901, the Czech Republic (Bohemia, Silesia) in 1843, 1864, 1877 and 1893, Poland in 1900, Austria (Tyrol, Burgenland, Lower Austria) in 1893, 1937 and 1953, and Switzerland (Graubünden) in 1914 (Anonymous, 1877; Anonymous, 1843; Jackl and Hauck, 1864; Märker-Kohlfurt, 1900; Merz, 1914; Osterberg, 1859; Roßmaeßler, 1845; K vonTubeuf, 1893). The alder species affected include *A. glutinosa*, *A. incana* and *A. viridis*. It has sometimes been suggested that trees are predisposed to insect attack by unfavourable soil conditions (such as a lowering of the groundwater level) or by other weakening factors – such as hail (Anonymous, 1877; Anonymous, 1843; Bittmann, 1893; Sedlaczek, 1937). Symptoms comprise premature wilting of the foliage accompanied by a blackish discoloration of the leaves. Crown dieback can occur, and there may be scattered bark necrosis and callus formation. However, this necrosis never forms a continuous lesion from the stem base and

rarely is there any mention of a black liquid oozing out from holes made by *C. lapathi*. Similar symptoms are reported from trees attacked by a second common stem-mining insect, the goat moth (*Cossus cossus*) (Bittmann, 1893; Ratzeburg, 1851; Skokan, 1853).

Micro-organisms

A number of microfungi have been implicated in the dieback of stems and branches of alder. Two species of the *Diaporthales* are quite frequently noted as being associated with bark necrosis: *Ophiovalsa (Winterella) suffusa* and *Valsa oxystoma* (Hartig, 1894; Münch, 1927; Münch, 1936; Münch, 1937; Nijpels, 1900; Schwarz, 1928; Truter, 1947; von Tubeuf, 1893). However, with a few exceptions (Appel, 1904; Nijpels, 1900; Schwarz, 1928), most authors regarded these fungi as weak parasites, i.e. only causing the death of bark after predisposition of the host by some other factors such as frost.

During the period 1977 to 1987, the microflora of declining *A. incana* in a heavily polluted industrial area of Poland were investigated (Domanski and Kowalski 1987). Although the complex of symptoms is referred to as a dieback of crowns, leaves with symptoms of 'advanced atrophy', 'more or less uniformly distributed in the whole crown' were observed in addition to heavy fructification and even tarry spots on the stems. *Hypoxylon fuscum* was regarded as the main pathogen but it may be noted that there are many other diseases of broadleaved trees which have been attributed to species in the genus *Hypoxylon*, only for it to be discovered later that the *Hypoxylon* is merely a secondary agent. It is a characteristic of *Hypoxylon* spp. that they develop from endophytic inocula in the xylem once the host tree has suffered serious damage from some other cause. At present, the possibility remains that this disease was caused by *Phytophthora*.

Reports of alders suffering from root rot are rare (Eisenmenger, 1894; Pfeil, 1859), and so far no descriptions of crown symptoms on trees suffering from root diseases have been found. However, Peace (1962) considered that root pathogens of the genus *Armillaria* occurred quite commonly on alder. During the course of the Concerted Action, mycelium of *Armillaria* was not uncommonly found on trees that had been affected by Phytophthora disease. Much more rarely, basal lesions attributable to *Armillaria* alone were found at woodland sites. Tarry spots were present on these lesions (Figure 3.1) but no crown symptoms were apparent.

Figure 3.1 Tarry spots on *Alnus incana* infected with *Armillaria* (J. N. Gibbs).

In Tuscany, alders suffered from a decline during the 1950s (Moriondo, 1958). Descriptions of crown symptoms are lacking, but the most striking feature of the disorder was the presence of limited bark necroses on the stem with associated tarry spots on the surface. These bark necroses developed into cankers with more or less strong callus production, which resulted in longitudinal cracks in the bark. The author concluded that the primary disease agents were bacteria infecting the trees via lenticels. Support for this interpretation came from later research in which cankers were incited on 2-year-old *A. glutinosa* and *A. cordata* by inoculation with a bacterium derived from symptomatic trees (Surico and Mugnai, 1992). This bacterium was subsequently described as a new species of *Erwinia*: *Erwinia alni* (Surico *et al.*, 1996).

Mortality in both common and Italian alders was reported from Southern Italy in the 1990s. Here, mycoplasma-like organisms (MLOs) were isolated from symptomatic trees and these were believed to be the dominant pathogenic factor (Marcone and Ragozzino, 1994; Marcone *et al.*, 1994). Leaves were sparse, abnormally small and fell prematurely. Sprouts appeared on the stems in connection with cankers. Longitudinal cracks were observed particularly at the stem base, which the authors interpreted as a consequence of reduced frost resistance. Dieback of branches also occurred. From these descriptions the alder Phytophthora cannot be entirely excluded as a hidden cause, since there was a more or less simultaneous crown decline in combination with bark cankers. However, the description of the symptoms is sufficiently detailed for it to be assumed that if bark necroses had their origin mainly at or below the stem base, this would have been mentioned. As far as the pathogenicity of MLOs is concerned, it should be noted that they have been detected both in healthy alders and in trees displaying a variety of dieback and decline symptoms (Lederer and Seemüller, 1991).

Abiotic damage

Drought periods in summer have been regarded as a primary cause of alder mortality from as far back as 1825 (Germany, Lower Saxony: Stassen and Behrisch, 1925). Later reports originated from Austria in 1889 (Althann, 1889), Germany/Poland (Pomerania) in 1904 (Appel, 1904) and Switzerland in 1911 and 1930 (Aubert, 1914; Fankhauser, 1930). In Pomerania, drought stress in winter, together with several other factors, was seen as part of a complex phenomenon. Trees suffered at the very least from a decrease of increment, and more often from dieback. Sometimes this took the form of a downward-extending bark necrosis on the stem.

Drainage measures and the canalisation of rivers have frequently been blamed for damage to riparian alder stands in Europe. Reports relating to this come mainly from the silvicultural literature, and the descriptions of symptoms are consequently scant. In 1833, riparian stands along the river Ems in Eastern Friesia were reported to have suffered severely from its canalisation (Germany: Müller, 1833) with large numbers of trees dying. In 1891 flooding of the river Oder in Poland destroyed large stands of common alders (Schmidt, 1892). In 1892 a violent flooding in the Danube deposited a thick layer of mud on the lower parts of the stems of grey alders, and this is reported to have resulted in a rotting of the roots, a decrease of increment, and the death of many trees (Eisenmenger, 1894). Between 1912 and 1932 the climate in northern Germany was characterised by heavy rainfall – floods occurred frequently and the groundwater level rose – so that young alder trees declined due to oxygen deficiency (Gassert, 1934).

Acute water shortage following the establishment of a well-field for drinking-water extraction was considered to be the cause of a sudden crown dieback in *A. glutinosa* that occurred in 1995 at a site in northeast Scotland investigated by S.C. Gregory and G. McGowan

(unpublished report). They observed tarry spots associated with isolated patches of necrotic bark on the stems of affected trees.

In a short note about drought damage on grey alder in the riparian forest of the river Danube in Austria, reference was made to 'burning spots' on the southern side of the trees. After seven weeks without rain and temperatures up to 40°C (Althann, 1889), these enlarged progressively, resulting in a dieback of the upper parts of the crown. It is very likely that this description refers to sun-scorch, which is a common phenomenon on trees with smooth thin bark.

Spring frosts during the development of the current year's shoots often result in injury, and sometimes older tissues are also affected. Since early in the 19th century, it has been recognised that abnormally mild periods during the winter can predispose alders to this kind of damage (e.g. Augst, 1903). Dieback occurred across the whole of Silesia (Germany/Poland) after a spring frost in 1914 (Rockstroh-Karmine, 1915). Reports of winter cold damage are rare in central Europe and limited to extremely cold winters, since all three non-mediterranean species of *Alnus* are reported to be quite resistant to this type of injury (Meyer ,1901). However, the formation of frost-cracks has occasionally been described (Beling, 1888).

A striking instance of crown dieback and tarry spot production in a roadside planting of *A. glutinosa* examined in the UK in 1995 proved to be due to 'backflash' through root grafts of the herbicide glyphosate, which had been applied to the stumps of adjacent trees to prevent resprouting (J.N. Gibbs, personal communication). In field studies conducted during the course of the Concerted Action, it has, not surprisingly, often been noted that crown symptoms similar to those caused by the Phytopthora disease can result from mechanical damage that disrupts the normal physiological processes of the tree. A common example occurs when the stem of a tree is girdled with fencing wire.

Complex damage

The 'Erlensterben' phenomenon

'Erlensterben' (alder decline) was a widespread problem, of no apparent cause, which principally affected *A. glutinosa* in mainland Europe at the end of the 19th and beginning of the 20th centuries. Commonly, the condition was reported as occurring on planted trees which grew outstandingly well during their first 10 to 12 years and fruited early in their development. Then between the 13th and 20th year, height growth of the trees decreased and the crowns began to die back. Bark lesions, notably brown in colour, developed from the crown to the stem base and these bore numerous fructifications of *Valsa oxystoma*. According to Münch, the first author to collect reports, the history of 'Erlensterben' can be traced back to 1865 when efforts to grow common alders in northern Germany (Mecklenburg) were unsuccessful. In a number of reviews of the available evidence, he also concluded that this failure could not be explained satisfactorily by known climatic, edaphic or biotic factors (Münch, 1927, 1935, 1936, 1937). After the turn of the century, cases of 'Erlensterben' were reported not only from Germany but also from Belgium, The Netherlands and Denmark (Hermansen, 1929; Nijples, 1900; Truter, 1947).

From the frequent observation that alders of natural origin growing near the dying trees were not affected by this disease, it was thought that the planted alders might have originated from unsuitable provenances (Bansi, 1924). Münch investigated the history of the alder plantations in Germany and came to the conclusion that most of the plant material had come from one small area in Belgium characterised by a mild Atlantic climate, and consequently was not adapted to the hard winters that characterise the more continental conditions of Central Europe (Urosevic, 1963). While this view gained general acceptance, it should be noted that Münch himself did not

think that every plantation failure should be attributed exclusively to unsuitable planting material. Along with others (e.g. Kremser, 1957) Münch was concerned to point out that the situation at any one site might be quite complex with other factors such as water level changes playing a part. Whatever may have been the cause, there is no doubt that 'Erlensterben' as defined by Münch and later workers appears to have been purely a crown dieback phenomenon. There is a complete lack of any reference to symptoms which would implicate the involvement of pathogens capable of attacking the roots or stem base (Kremser, 1957; Weiss, 1965).

Alder dieback in Northern Britain

There has been considerable concern over the health of *A. glutinosa* in Northern Britain during the last 15 years (Figure 3.2). The symptoms of affected trees were described by Gregory *et al.* (1996) as follows. Branches (both large and small) showed bark lesions and dieback. In some cases, only a single branch was damaged but usually several were affected and bark necrosis extended into the main stem. It was not uncommon for symptoms to appear simultaneously in two or more small branches on an otherwise healthy tree. Dead bark on infected branches and stems might be visible as depressions in the bark surface but there were often extensive tongues of active necrosis beyond these, detectable only by cutting into the bark. Within the larger lesions, there were often patches of split or cracking bark. The overall appearance was one of a condition that was capable of progressive development within the tree. It appeared that this might be quite rapid as, during summer, tongues of recently killed bark on larger branches or main stems frequently included still green and active side branches. Bark necrosis was usually associated with purple–brown staining of the wood and this might extend under live bark well beyond the area of outward visible symptoms.

Figure 3.2 Dieback of alder alongside a stream in the west of Scotland (J. N. Gibbs).

Symptoms on individual trees were not followed systematically from year to year, but examination of a large number of affected trees strongly suggested that the disorder was a progressive one, from which many trees might die. However, in localities where dieback was evident, trees were often found with a dead disintegrating top but a healthy new crown at the base developing from epicormic shoots. Such trees suggested that recovery might be possible, though perhaps not commonly. It was not unusual to find vigorous live trees that were close to dead trees and trees with serious dieback. Often skeletons of a few dead trees were almost hidden in thickets of live ones, but the reverse situation was also encountered with only occasional live trees interspersed among numerous dead ones. It was reported that in a few locations, alder had been virtually eliminated along significant stretches of river bank.

Gregory *et al.* (1996) considered the possibility that one or more living agents might be involved. A number of fungi were found fruiting on dead bark or were isolated in the laboratory from dying bark and stained wood. These fungi included *Ophiovalsa suffusa*, *Melanconis alni*, *Cryptosporiopis* sp. and *Inonotus radiatus*. In addition, two unidentified species were commonly isolated from lesions. The consistent association of certain species with the dying bark suggested that these fungi might be pathogenic, even if only weakly so. However, a small scale experiment in which *Cryptosporiopsis* sp. and the two unidentified species were inoculated into the main branches of vigorous young alders failed to incite any symptoms (S.C. Gregory, personal communication).

Although the incidence of damage in affected areas and the severity of symptoms on individual trees might suggest the presence of an aggressive disease, it was hard to reconcile this explanation with the distribution of the damage along certain rivers. The fact that in some valleys a stretch of river bank had high and apparently long standing mortality whereas a few kilometres downstream there was little or no visible damage, suggested that site factors might have an overriding influence on the development of the condition. However, it was noted that the pattern of symptoms observed upon damaged trees was consistent with the class of diseases which are caused by latent pathogens (Gregory *et al.*, 1996). Such latent pathogens frequently establish themselves within healthy hosts, causing little or no disease until the defences of the tree are reduced by stress or injury. The strong influence of site on the severity of dieback would also be consistent with the action of a latent pathogen, with trees at different locations encountering different levels of stress. Studies on the problem are continuing and a series of long-term observation plots has been established.

Damage to shelterbelt alders in The Netherlands

In studies conducted since the start of the Concerted Action, two localised cases of alder dieback were attributed to the combined effects of herbicides and members of the Oomycete genus *Pythium* (C. van Dijk and J. de Gruyter, personal communications). At one location, Zaltbommel, visual symptoms on four *A. glutinosa* trees closely resembled those caused by the Phytophthora disease, including sparse, yellowish and small leaves and tarry spots at the stem base. However bark lesions at the site of the tarry spots were small and not typical. All attempts to isolate *Phytophthora* from these lesions failed, but isolation from soil-root samples yielded abundant *Pythium* 'group P'. In biotests these *Pythium* isolates caused root killing on young alder plants. The next growing season two of the trees were dead, while the others recovered slowly. At this location there was clear evidence of the effects of herbicide application to adjacent meadows and it seemed likely that excessive rates had been accidentally applied and that these had resulted in damage to the trees.

The second case concerned a young orchard shelterbelt at Zetten comprising about 600 m of *A. cordata* with typical leaf symptoms, but no tarry spots. Here too isolations from stem bark failed, but *Pythium* 'group P' and *P. sylvaticum* were isolated from root fragments and proved capable of causing root damage to seedlings of *A. cordata*. Frequent treatment of the orchard soils with the herbicide glyphosate had occurred since 1997. The symptoms developed irregularly over three years and resulted in mortality of 5–10% of the trees. Shelterbelts of black alder in the same orchard, of the same age and subject to the same herbicide regime, were free from symptoms, pointing to a differential sensitivity of the two alder species to the damaging agents (C. van Dijk and J. de Gruyter, personal communications).

Conclusions

In this chapter, a wide variety of different types of disease are described. The causes of some of them have been elucidated; the causes of others are largely hypothetical. Some, such as the dieback currently affecting alder in Northern Britain, remain under investigation. However, it can be concluded with some confidence that there are no reports of disease before the 1980s that are strongly suggestive of the *Phytophthora* disease. This is consistent with the information presented in Chapter 4 which indicates that the disease only became widely apparent in the 1990s, with evidence in a few places, such as the UK and Austria, that it was present back into the 1980s.

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Note that in some of the older references, initials of authors' names were not used.

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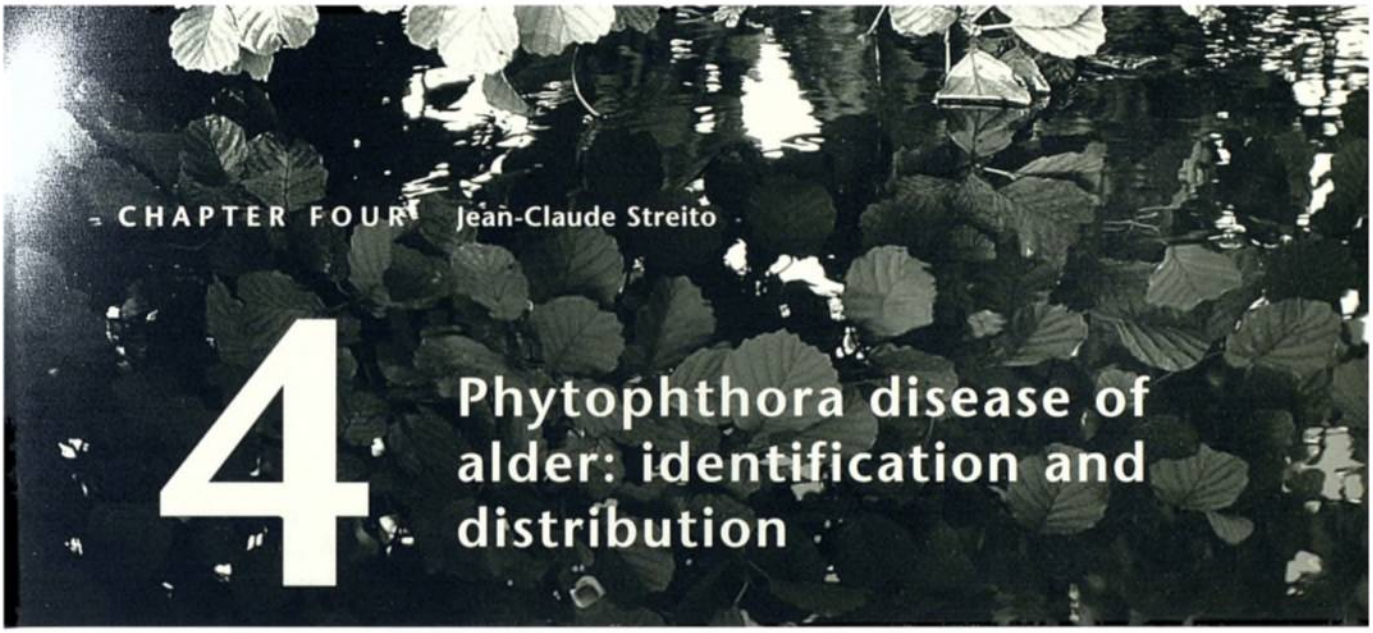


CHAPTER FOUR Jean-Claude Streito

4 Phytophthora disease of alder: identification and distribution

Introduction

When first reported from the UK (Gibbs *et al.*, 1994; Gibbs, 1995), the Phytophthora disease of alder was said to be characterised by the following features:

- leaves were abnormally small, yellow and sparse (Figure 4.1);
- dead roots were present and strips of dead bark, extending up from the collar level, occurred on the stems. These stem lesions were marked externally by the presence of tarry or rusty coloured exudations (Figure 4.2).

A fungus with some similarities to *Phytophthora cambivora* was regularly isolated from the diseased trees (Brasier *et al.*, 1995). Subsequently, Brasier *et al.* (1999) showed that the fungus comprises a range of hybrids with distinct behavioural and morphological properties (see Brasier, Chapter 5). To cover this situation, the term 'alder Phytophthoras' has been used.

Unlike some pathogens, Phytophthoras can be quite difficult to isolate from diseased necrotic material. Thus a requirement of the Concerted Action programme was to exchange information on the most effective methods of isolation. A summary of the experience gained by those involved in the Concerted Action is presented in this chapter. As the work progressed, some isolates of other *Phytophthora* species were obtained and data on these are also reported. A chronology of records of the alder Phytophthoras is presented, together with a distribution map, and the chapter concludes with an assessment of the characteristic symptoms of the disease across its range.

Figure 4.1 *Alnus glutinosa* beside a river in southeast England, showing crown symptoms of Phytophthora disease.

Figure 4.2 Basal lesion on stem of *Alnus glutinosa* as revealed by cuts in the bark. The tarry and rusty exudations are very conspicuous.

Procedures for isolation and detection of *Phytophthora*

The presence of competing saprotrophic microorganisms can make it difficult to isolate Phytophthoras. Over the years, a variety of procedures has been developed to overcome this problem. Some rely on the selective qualities of living tissue to 'bait' for the fungus while others depend on the use of special synthetic media (Erwin and Ribeiro, 1996). These procedures have provided the basis for studies on the alder Phytophthoras.

Given the characteristics of the disease, the prime material for isolation has been diseased bark, and the techniques for working with this material have received considerable attention. However, there have also been studies on soil samples and an attempt to isolate the fungus from river water in areas where the disease is well established.

Isolation from bark lesions

Isolation procedures used in the UK (Brasier *et al.*, 1995; Gibbs, 1995) have been based on the 'green apple' method in which samples of necrotic bark are inserted into cuts made in unripe apples (Brasier and Strouts, 1976). After incubation of the apples, sub-cultures onto agar are made from any lesions that are characterised by a firm texture and a brown to orange colour. Other partners in the Concerted Action have made extensive use of direct plating of bark fragments onto agar. Several selective media have been used which contain various antibiotics, such as benomyl and hymexazol. The composition of one of them has been published (Streito *et al.*, 2002a). Alternatively, non-selective media such as corn meal agar, cherry agar and carrot agar have also been used (Streito *et al.*, 2002a; H. De Gruyter and C.J. van Dijk, unpublished; S. Werres, Records of the CA). In one study, H. De Gruyter and C.J. van Dijk (Records of the CA) compared plating on cherry agar with a

green apple technique and found both methods to be equally effective.

The selection of the best sample material for isolation has aroused considerable debate. Not surprisingly alder Phytophthoras have readily been obtained from fresh active inner-bark lesions that are contiguous with healthy tissues. Isolation success from older drier material has been much more variable although workers such as T. Jung (Records of the CA) have reported good success. C. Olson (Records of the CA) was able to isolate the fungus at the riverside, by placing fragments of bark, without any surface disinfection, immediately onto a selective medium. However, the more general practice has been to transport the material to the laboratory. Polythene bags are usually used but T. Jung (Records of the CA) immerses the pieces of bark in sterile water for transportation. Undoubtedly, rapid processing of the samples is desirable, although Streito *et al.* (2002a) have shown that isolation is possible after samples have been stored at 7° ± 4°C for one month. Dehydration is the most likely cause of isolation failure and this can often be prevented if the sample is kept sufficiently moist during storage, for example by wrapping in damp paper.

In UK studies, fragments of bark are washed for 24 h in running water before being inserted into the apple (Brasier *et al.*, 1995). Werres (Records of the CA) has employed a similar approach before fragments are placed on carrot agar. Streito *et al.* (2002a) describe how the outer bark is cut away in the laboratory and the sample is then washed for a few minutes under tap water, before being dried with filter paper. The whole surface is disinfected quickly with 70% ethanol and dried again under sterile conditions. There is general agreement that the drying process limits bacterial growth, and so is critical to success.

Isolation is possible throughout the year. However, success is generally greater using samples obtained in the second half of the year rather than in the first. Streito *et al.* (2002a) reported that in 1997 active lesions were not observed in the northeast of France before July and the best period of sampling was in September and October. However, in 1998 fresh bark killing was observed as early as May and successful isolations were made at this time (J-C. Streito, Records of the CA). This high success rate continued until the end of October. This suggests that the onset of activity by the pathogen can vary from year to year and has an influence on the likelihood of successful isolation.

Isolation from soil

Attempts to isolate the alder Phytopthoras from soil samples have sometimes been made in the course of surveys for the disease and sometimes as part of investigations into its biology. Procedures have closely followed those used for other Phytophthora diseases (see for example Erwin and Ribeiro, 1996; Jung *et al.*, 1996). In brief, soil samples can be inserted directly into apple baits or can be subjected to a dilution series before being plated onto selective media. Alternatively, water can be added to the soil sample and suitable living plant tissue floated on the surface to act as a bait for any *Phytophthora* that is present. Lesions that develop on the bait tissue are then sub-cultured onto agar. At the outset of the Concerted Action it was not clear what kind of plant tissue might be suitable for the alder Phytophthoras and experimentation was encouraged. Alder leaves were found to be unsatisfactory although J. Delcan and C.M. Brasier (see Brasier, Chapter 5) found that *Phytophthora gonapodyides* was frequently isolated. T. Jung (Records of the CA) found that oak leaflets, previously used for the isolation of *P. quercina* (Jung *et al.*, 1996, 1999, 2000), provided a suitable bait. Rhododendron leaves were also used successfully (Themann and Werres, 1998; S. Werres and J-C. Streito, Records of the CA).

In the Netherlands, C. van Dijk (Records of the CA) adapted a technique that had been developed to detect the presence of the symbiotic actinomycete *Frankia*. Soil suspensions were added to 6-week-old alder seedlings grown in hydroculture on Hoagland nutrient solution (van Dijk and Sluimer, 1994) and root infections due to *Phytophthora* could be detected within 1 and 3 weeks. The fungus could then be isolated into pure culture by plating samples of the diseased rootlets onto a selective medium.

Isolation from river water

In northeast France an attempt was made to use freshly cut alder twigs to trap alder Phytophthoras from river water (Streito *et al.*, 2002a). Twigs *c.* 1 cm in diameter and *c.* 10 cm in length, joined together to form a raft, were moored to the bank and left to float on the surface of the river. The rafts were removed after 1 week in summer and 4 weeks in winter and isolation attempts made from any necrotic areas of bark that were visible on the twigs. However, less than 1% of necrotic bark fragments yielded alder Phytophthoras.

Detection by molecular diagnostics

For some years there has been interest in the use of molecular diagnostics for *Phytophthora* species but the early molecular probes were of relatively little value because of their lack of specificity. There is no doubt that a DNA-based molecular identification method would be of use in the study of the Phytophthora disease of alder, particularly if it provided an opportunity to confirm the presence of the pathogen in situations where isolation was not possible. Such a technique might also be useful in the detection of the fungus in soil or river water.

Recent developments with other *Phytophthora* species have been more encouraging in this respect. For example, good results have been obtained in detecting the presence of *Phytophthora cactorum* in infected plant material, including the wood and bark of *Betula*, using species-specific PCR primers (Lilja *et al.*, 1996). This work is now being developed to determine whether it can be used to detect the presence of the fungus in soil (A. Lilja, personal communication). Primers (Schubert *et al.*, 1999) and a suitable protocol (Nechwatal *et al.*, 2001) have also been developed to detect *P. cambivora, P. citricola* and *P. quercina* present in fine roots and rhizosphere soil of oak (*Quercus robur*) and beech (*Fagus sylvatica*).

In general the prospects for developing an effective molecular identification protocol for the alder *Phytophthora* are good. The ITS sequence data for the fungus, published by Brasier *et al.* (1999) and lodged in the GENBANK database, provide sufficient information to make it feasible for a specific set of PCR primers for the fungus to be constructed.

Phytophthora species associated with disease in alder

The vast majority of Phytophthoras isolated from diseased alder have corresponded to the alder Phytophthoras as described by Brasier *et al.* (1995). Records of alder Phytophthora disease across Europe are provided in the next section. However, there have also been a few records of other *Phytophthora* species on *A. glutinosa* and these are shown in Table 4.1. The most frequently recorded are *P. citricola, P. cactorum* and *P. gonapodyides.*

P. citricola has been isolated from a basal lesion on a large tree growing on a site subject to inundation by brackish water in eastern Sweden (J. Stenlid, Records of the CA). It has also been isolated from basal lesions on trees in Lower Saxony (S. Werres, Records of the CA) and Bavaria (Jung *et al.*, 2000). In France *P. citricola* was isolated from necrotic roots in 1999

Table 4.1 Records of other Phytophthoras isolated from *Alnus glutinosa*

Species	Host tissue	Notes on site	Country	Citation
P. citricola	Stem base		Germany	S. Werres, Records of the CA
	Stem base	Plantation	Germany	Jung *et al.*, 2000
	Stem base	Subject to flooding with brackish water	Sweden	J. Stenlid, Records of the CA
	Roots		France	J-C. Streito, unpublished.
	Stem base		France	J-C. Streito, unpublished.
P. cactorum	Stem base	Nursery	Finland	Lilja *et al.*, 1996
	Stem base			J-C. Streito, Records of the CA
P. gonapodyides	Fine roots	Pond-side	Denmark	Thinggaard, 1996
	Fine roots	Pond-side	Denmark	K. Thinggaard, Records of the CA
	Stem base		UK	J.N. Gibbs, Records of the CA
	Main roots		Germany	T. Jung, unpublished
P. megasperma	Stem base		Germany	S. Werres, Records of the CA
P. syringae[a]	Stem base	Plantation	Germany	Jung *et al.*, 2000
Phytophthora	Stem base		France	Unpublished, record of DSF Sud-Ouest
Phytophthora	Stem base		Germany	S. Werres, Records of the CA

[a]To be named *P. pseudosyringae* (T. Jung, personal communication).

and from the base of an old tree in 2000 (J.C. Streito, unpublished). In addition, this fungus has been isolated at high frequency from the soil of a low vitality stand of *A. glutinosa* in a peat bog area of relative high salinity in the Netherlands (van Dijk, personal communication). Isolates from this source proved to be extremely pathogenic when added to the root environment of alder seedlings in biotests.

P. cactorum is well known as a pathogen of *Malus* and *Pyrus* and has been reported from over 83 genera of herbaceous and woody plants (Ribeiro, 1978). It has been found in stem base lesions on nursery plants of alder in Finland (Lilja *et al.*, 1996) and in necrotic stem bark in France.

P. gonapodyides was isolated from the fine roots of a young alder at the edge of a pond in Fyn, Denmark in 1996. The tree was showing crown symptoms suggestive of the Phytophthora disease but there was no evidence of a stem lesion or of tarry spots (Thinggaard, 1996). In 1997 a second isolation of this species was made from a moat-side tree in Funen. In the UK, *P. gonapodyides* was isolated from a stem lesion on a riparian tree of *A. glutinosa* in 1996. The lesion extended to more than 2 m from ground level and showed abundant tarry exudation but the leaves of the tree were of a normal size and colour (J.N. Gibbs, Records of the CA). The same fungus was also isolated from bark lesions on main roots of a diseased riparian *A. glutinosa* in Bavaria (T. Jung, unpublished). *P. gonapodyides* is a species complex that is commonly reported from dead plant debris in ponds, rivers and damp soil. It is usually considered to be a saprotroph (Erwin and Ribeiro, 1996) and may not have been the causal agent of the disease in these trees.

Records of alder Phytophthoras in Europe

When the Concerted Action began in March 1998, the pathogen was known to be present in six countries: the United Kingdom, The Netherlands, Germany, Sweden, France and Austria. It was also thought likely to be in Denmark, although the symptomatic trees had only yielded *P. gonapodyides* (see pages 28–29). During the course of the Concerted Action, and aided by the exchange of information that it offered, the presence of the pathogen was confirmed by Partners working in Belgium and Ireland. Records also came in for Italy and Hungary. Despite rigorous work, alder Phytophthoras were not found in Finland and Norway, and their presence could not be confirmed in Denmark.

The order adopted here for the countries is based on the date on which an alder Phytophthora was first isolated from a diseased tree and recognised as such. Only limited information on the distribution of the various variants is presented: more details on this subject can be found in Brasier (Chapter 5).

Figure 4.3 Distribution of Phytophthora disease in the UK in 1995 showing rivers on which the diseased trees had been seen. Circles indicate the locations of affected trees. Arrows show those locations at which an alder Phytophthora was isolated.

The United Kingdom

The first isolation and recognition of an alder Phytophthora came from the UK in the summer of 1993 (Gibbs *et al.*, 1994). By the end of 1994 it was known to be widespread in the southern half of the country and a map showing river systems on which the fungus has been isolated was published by Gibbs (1995), and is reproduced here as Figure 4.3. Within a few more years it had been recorded in most parts of England and Wales. The fungus was first recorded in Scotland in 1996 but the trees, on the river Spey, were also suffering from dieback due to extreme water shortage (Gregory and McGowan, 1996), and it was not until 2000 that typical symptoms of the disease were observed (J. N. Gibbs, Records of the CA). These occurrences concerned several other major rivers in northeast Scotland and also a small river in the northwest, the Allt Duirinisch, which flows only a few kilometres from its source to the sea.

Although the disease was only recognised in Britain in 1993 there is no doubt that it was present for some years before that date. Records of the Disease Diagnostic and Advisory Service of Forest Research (the Forestry Commission Research Agency) show that Phytophthora disease was diagnosed in 1987 in a mixed planting of *A. cordata, A. glutinosa* and *A. incana* in Bedfordshire, east England. At that time attempts to isolate a Phytophthora were unsuccessful, but at a later visit to the site in 1994, an alder *Phytophthora* was isolated from a diseased *A. cordata* (Gibbs, 1995).

The Netherlands

An isolate of alder Phytophthora, obtained in 1992 from *A. glutinosa,* was included in the UK studies carried out in 1994 (Brasier *et al.*, 1995). However, analysis of the records of the Netherlands Plant Protection Service indicate that a fungus, that may well have corresponded to the alder Phytophthora, was isolated from *A. cordata* in a shelterbelt as early as 1983. Both the 1983 and the 1992 isolates were obtained from symptomatic tissue at the stem base but there is no further information on the condition of the trees. In the autumn of 1995, C.J. van Dijk (unpublished) isolated the fungus from soil in the De Wieden Nature Reserve, during a study of Pythiaceous root-rot pathogens in various natural alder stands. None of the trees in the vicinity showed symptoms that would be considered typical of Phytophthora disease.

During the next few years a wide variety of diseased alder stands were examined. Although several other *Phytophthora* species were occasionally encountered (see previous section), no alder Phytophthora was isolated. However in 2000, hundreds of trees with classic symptoms were reported on flood plains of two brooks flowing into the River Maas in the south of the country by H. de Gruyter and C. van Dijk (Records of the CA). Isolations from representative bark material readily yielded the pathogen. On both streams the trees had been adversely affected by an artificial raising of the water levels. It should be noted that the form of the pathogen isolated from these sites was different from that obtained from De Wieden (see Brasier, Chapter 5).

Germany

The first records of the disease came from northern Germany when Hartmann (1995) reported isolating the fungus from two alder plantations in the Luneberger Heide. Classic visual symptoms were observed in 1998 in the Marschland of northern Germany by S. Werres (Records of the CA) and, in the same year, information was sought from foresters across the country (Werres,1998). This resulted in details and soil samples being received from 45 declining alder stands and the alder Phytophthora was isolated from *c.* 7% of these. Site visits to some of the stands followed, and in 2000 the pathogen was isolated from a tree in the Spreewald, a remarkable protected region southeast of Berlin where large alders of great economic value grow in an area of about 470 km^2 intersected by a network of canals. It was also readily isolated from severely affected riparian alder in several other parts of the country (S. Werres, Records of the CA).

Also in 1998, B. Metzler working in Baden Wurttemburg reported isolating the alder Phytophthora from basal lesions on trees at two sites near the Rhine, both of which were subject to seasonal flooding (see Schröter *et al.*, 1999). In Bavaria a major study of the disease was initiated in 1999. By July 2001 the alder Phytophthora had been isolated from 41 mature alder stands and 17 alder plantations across the region, and typical symptoms been seen at many others (Jung *et al.*, 2000 and unpublished).

Sweden

The disease was discovered in western Sweden by C. Olsson (Records of the CA) in 1996 on the banks of the River Säveån. The alder Phytophthora was isolated from a number of sites: one within the city of Gothenburg itself, the others 8–10 km upstream, and one further east at the city of Alingsås. In 1997, the pathogen was isolated from necrotic roots on a sample of 1-year-old alders removed from a nursery in Ljungbyhed in southern Sweden. The nursery takes its irrigation water from the adjacent river Rönneå. The nursery was inspected during 2000 by the Plant Inspectorate (Swedish Board of Agriculture). Samples of alder plants were tested using the *Phytophthora*

DAS ELISA test and isolation onto agar but were found to be healthy. Alder plants from another nursery close to the city of Kalmar, near the east coast of Sweden, were tested by the same procedure and also found to be healthy (C. Olsson, unpublished data).

In 1998 the disease was also found on the edge of Lake Stensjön on the outskirts of Gothenburg. This lake is on a different river system to Säveån and while all the isolates from the Säveån are of the so-called Swedish variant of the fungus, that from Lake Stensjön is the so-called 'British' variant' (see Brasier, Chapter 5). Despite much searching, the disease has not been found elsewhere in the country.

France

A full account of the situation has been provided by Streito *et al.* (2002b) and a map from that study is shown as Figure 4.4. An alder Phytophthora was isolated in 1996 along the river Moselle near Nancy, by staff of the Laboratoire National de la Protection des Végétaux, and in the Landes near Bourdeaux, by C. Robin of the Institut National de la Recherche Agronomique. It quickly became clear that the pathogen was very widely distributed in the northeast of the country. By 1999 it had been isolated from more than 110 different sites and it was known that all the main rivers and most of the tributaries were affected.

The record from the Landes came from the banks of the lake of Leon where mortality in alder had been observed as early as 1991. Also in western France, mortality had occurred for some years along the Charente River. In Brittany, where dieback in alder had been reported for some years, the fungus was isolated in 2000. In southeastern France, one case has been confirmed near Avignon along the River Rhône. Some alder declines have been recorded in the Massif Central and in two other regions of southern France (Lot and Tarn) but the pathogen has not been isolated.

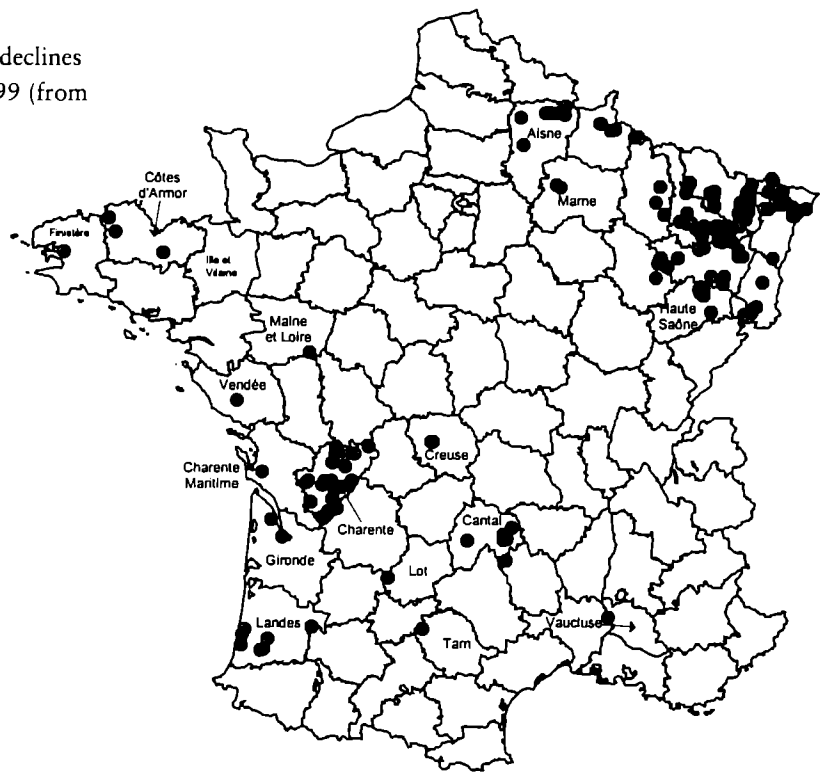


Figure 4.4 Distribution of alder declines in France between 1996 and 1999 (from Streito *et al.*, 2002b).

Austria

The presence of Phytophthora disease in alder was confirmed for the first time in 1996, when the fungus was recorded from two stands of *A. glutinosa* in Upper Austria. In 1998 it was isolated from diseased *A. incana* on the Danube, from *A. glutinosa* on the border between Austria and Czechoslovakia, as well as from another stand of *A. glutinosa* in Upper Austria, where difficulties with isolation had delayed confirmation for two years. The sites at the Czech border (riparian stands on the river Thaya and its tributaries) are of special interest, since what are now known to be symptoms of the disease had been observed as early as 1986. In the early 1990s no diseased trees could be found but in 1998, the symptoms reappeared and the alder Phytophthora was successfully isolated (Cech, 1997; Cech and Brandstetter, 1999).

In a few localities in Upper Austria, the disease has had a considerable impact but generally it is rare in this area, and even when present, does not seem to be progressive (see Gibbs *et al.*, Chapter 6). However, in 1999, the pathogen was identified from *A. glutinosa* in Styria, close to the border of Burgenland, an area in which dense alder populations occur and where there are many slow-flowing rivers. In 2000 it became obvious that numerous stands of black alder in the Burgenland itself, notably the river systems of Pinka and Stögersbach, were suffering from the disease and the fungus was isolated from four locations. At present there are no reports of the disease from the *A. incana* stands that line the numerous white-water rivers of the Austrian Alps. However in December 2000 an alder Phytophthora was isolated from *A. incana* along the River Inn in Tyrol. These trees had been suffering from high levels of stagnant water since the construction of a power station two years earlier (T. Cech, Records of the CA).

Belgium

Although interest in the disease was roused by reports of symptomatic alders in the mid-1990s, it was not identified in Belgium until 1999 (Cavelier *et al.*, 1999). In that year, assessments showed that the health of alders had deteriorated dramatically along many rivers when compared to their condition two years previously. Decline was most notable on the Sûre, the Ourthe, the Mehaigne and the Vierre. Isolation of the pathogen was successfully carried out in September 1999 from *A. glutinosa* in a plantation established on wet ground intersected by drains linked to the River Dyle. In 2000 it was also obtained from two sites on the river Salm in the Ardennes (H. Claessens, Records of the CA).

Ireland

Dieback of alder has been known to occur commonly on some Irish rivers and isolation attempts were made from material from many sites during the late 1990s. However, it was only in November 1999 that the fungus was successfully isolated from a tree on the river Dodder in the city of Dublin (K. Clancy, Records of the CA).

Denmark

As described above, the original reports of Thinggaard (1996) do not now appear to have involved an alder Phytophthora. No records of the disease had been obtained as of 2000. Various investigations are in progress (L. Bodker, Records of the CA).

Finland

A major effort was made to publicise the symptoms of the Phytophthora disease and to check reports of suspicious trees. As of 2000, investigations had been made at 17 woodland sites. Trees showing stem lesions and tarry

spots, usually in association with some crown dieback had commonly been encountered, although it was noted that the leaves were not particularly small or yellow. Despite being able to take advantage of all the information on isolation techniques disseminated via the Concerted Action, Lilja (2000 and Records of the CA) has been unable to isolate the alder Phytophthora, or indeed any *Phytophthora* from the symptomatic trees.

Norway

The situation in Norway is similar to that in Finland. Trees with stem lesions (although not always basal ones) and crown dieback have been observed in a number of locations on the west coast of Norway and near Oslo. No *Phytophthora* has been isolated (I. Borja, Records of the CA).

Countries not involved in the Concerted Action

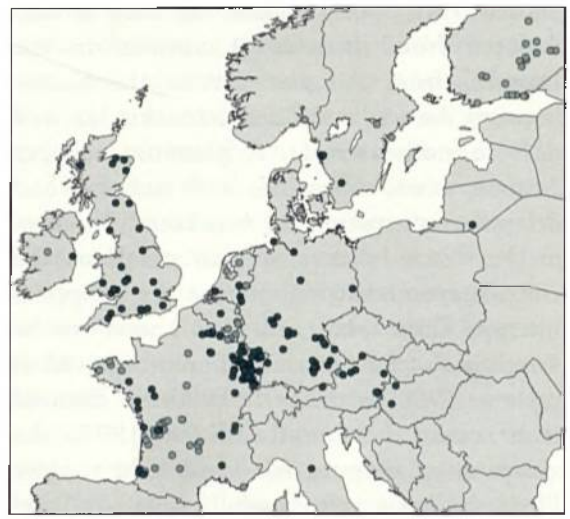
Among the other countries of the European Union, the disease is known only from Italy. In 2000 an alder Phytophthora was isolated from *A. cordata* seedlings in a nursery at Mugello in Northern Tuscany (Santini *et al.*, 2001). In 2000 it was also reported from Hungary (Iloni *et al.*, 2000). In Estonia symptoms suggestive of the disease have been seen in the oil-shale area (A. Lilja, Records of the CA). So far no serious attempt at isolation has been made. After the conclusion of the Concerted Action, an isolate of the pathogen was received from Z. Joraisiene in Lithuania.

Mapping the distribution across Europe

The information compiled during the course of the Concerted Action has been used to produce a composite map (Figure 4.5) which shows the known distribution of the alder Phytophthoras across Europe. Where a very large number of records have been collected in a relatively small area, only a proportion of them are shown. The map also shows sites in which despite the presence of trees with symptoms resembling Phytophthora disease, careful isolation work has failed to yield an alder Phytophthora.

Figure 4.5 Records of alder Phytophthoras across Europe.

- locations at which the fungus has been isolated
- locations where isolation attempts have failed to yield the pathogen



Records of Phytophthora disease in different alder species

Within a short time of the disease being recognised it was clear that it could affect all three European tree alders – *A. glutinosa, A. incana* and *A. cordata* (Gibbs, 1995). The great majority of records that have subsequently accumulated concern *A. glutinosa*. This is not surprising as it is the most common species and the one that is most closely associated with the riparian habitat (see Claessens, Chapter 2). New records on both *A. incana* and *A. cordata* have been made as indicated above. There are as yet no records for *A. viridis*. The disease has been found on young *A. rubra* in a riparian plantation (J. N. Gibbs, unpublished).

The symptomatology of the Phytophthora disease of alder reassessed

Towards the end of the Concerted Action, when a great deal of accumulated experience on confirmed cases of the disease had been gained over a wide range of sites, it became possible to reassess the original description of symptoms. It should be noted that there is insufficient information on the few cases of disease associated with other species of Phytophthora for the symptoms of these to be described separately.

Bark lesions

- **Inner bark.** Since the crucial damaging activity of the fungus is the killing of bark, the lesion in the inner bark is the key symptom of the disease – although on a large tree with secondary bark it is not always easily seen. If the inner bark is freshly exposed with a knife, the recently killed tissue will be reddish to purplish brown and will often be marbled or mottled. It contrasts strongly with the creamy colour of adjacent healthy inner bark tissue.
- **Bark surface.** On the bark surface, there will very often be tarry or rusty spots. These result from the death and fermentation of inner bark tissue and, where secondary bark is present, may only be seen in the fissures between the bark plates. As they age, they become dry and less conspicuous. Although they can persist for several years, they may be washed off the base of the tree if it is exposed to floodwater.
- **Adventitious roots.** One of the features of alder is its ability to produce adventitious roots from the stem in response to a dysfunction of the existing root system (see Claessons, Chapter 2). The occurrence of such roots can be a useful indication of the presence of a bark lesion further down the stem (see Figure 2.3).
- **Aerial lesions.** Although most lesions can readily be seen to have developed up the stem from the base, 'aerial' lesions can undoubtedly occur. An early example was provided from northeast France. Here, the alder Phytophthora was obtained from an isolated bark lesion on the lower branch of a tree (J-C. Streito, unpublished). Similar lesions have been found in other countries. Thus, a clear example was found at 50 cm from the base of a 2.5 m high tree on the banks of the river Dee in Scotland in 2000 (J.N. Gibbs, Records of the CA). It should be noted that such symptoms have only been found below points on stems that will have been under water at times of flooding.
- **Callus boundary.** Bark lesions commonly develop until the stem has been girdled and it is usually at this point that crown symptoms appear. However, this does not always happen. In some cases the lesion ceases to extend and its boundary is soon marked by the production of a roll of callus from the surviving cambium (Figure 4.6).

Figure 4.6 Inactive lesion at the base of an *Alnus glutinosa* coppice stool in Upper Austria. The tarry spots can still be seen but a strong roll of bark callus is present. The tree showed no crown symptoms (J. N. Gibbs).

- **Root bark.** Much less is known about the status of the fungus in root bark than in stem bark. However, there is no doubt that root lesions can develop independently of any stem colonisation (see Lonsdale, Chapter 7) and field investigations in the UK certainly suggest that crown symptoms can sometimes appear in trees in which all the necrotic bark is below ground level (J.N. Gibbs, personal communication).

Crown symptoms

- **Foliage colour, size and shape.** In the initial description (Gibbs, 1995), the diseased tree was said to be characterised by the presence of small, yellow and sparse foliage. In the UK, at least, these symptoms are very commonly encountered. In a 'classic' case there may well also be a scattering of brown leaves among the yellow ones and there is a tendency for all the leaves to be 'cupped' slightly, i.e. for the lamina to be bent upwards on either side of the main vein. While very similar manifestations of the disease have been observed elsewhere, the full assemblage of foliage symptoms is not always found. Thus, in both Sweden and Germany it has been noted that affected trees can show a marked reduction in leaf size without leaf yellowing.
- **Fruiting.** There is general agreement that heavy fruiting is an extremely common feature of the disease. This was not mentioned in the original description of symptoms but is a common phenomenon in trees under stress.

Prognosis/progression of disease

Although many trees die rapidly once the crown symptoms have appeared, this is not invariably the case. Sometimes, small strips of bark remain alive and continue to sustain parts of the tree. This growth is often feeble, taking the form of scattered epicormic shoots which may subsequently die. However, sometimes growth may be sustained for many years, with the leaves regaining their normal size and colour (Figure 4.7). Unless the history of such trees is known, it may not be evident that the original cause of the damage was Phytophthora disease. In a coppice stool sometimes one stem is sufficiently damaged by the disease for it to die completely while other stems may recover or indeed may never contract the disease. Even where all the stems in the stool die, new shoots may appear at the base and these can remain healthy, although they frequently contract the disease in their turn.

Figure 4.7 *Alnus glutinosa* alongside a canal in Wales, UK showing no active symptoms of Phytophthora disease in 2001: classic crown symptoms of the disease and a basal lesion were recorded in 1995 (J. N. Gibbs).

Conclusions

A variety of techniques can be used to isolate alder Phytophthoras from bark on diseased trees. Not surprisingly, fresh active lesions provide the best material, but some success has been achieved with bark that has been dead for some time. The fungus has also been isolated from soil around the roots of diseased trees and, to a very limited degree, by baiting from river water.

Alder Phytophthoras are now known from eleven European countries. In the UK, the country in which the fungus was first described, there is evidence for its presence in the 1980s. However, here as elsewhere, there was a large expansion in the disease during the 1990s. The expression of disease shows a strong similarity across the continent. The bark lesion is the key symptom and this is most commonly to be found at the stem base. On severely affected trees, crown symptoms are characterised by the presence of abnormally small sparse leaves that are often, but not invariably, yellow in colour. While alder Phytophthoras have comprised the great majority of isolates obtained from symptomatic trees, there have also been some records for other species, *P. citricola* being the most common. *P. gonopodyides* has also been isolated on a number of occasions although it may have been present as a saprotroph.

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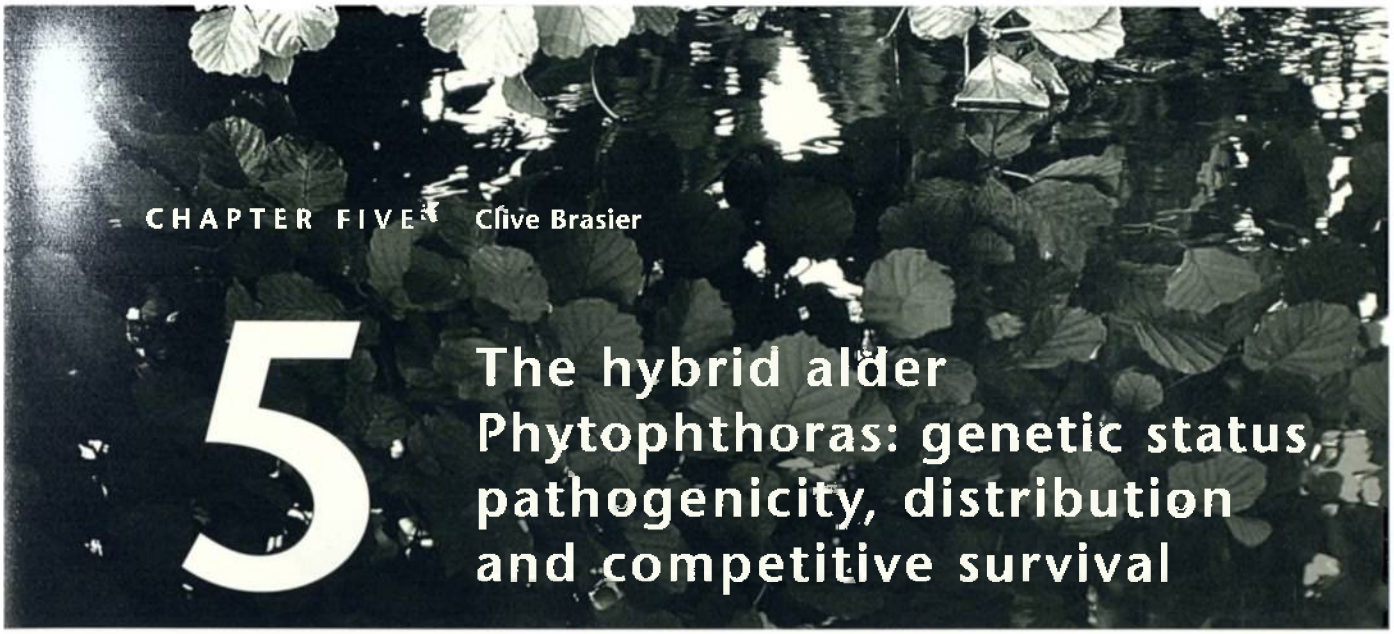


CHAPTER FIVE

Clive Brasier

5 The hybrid alder Phytophthoras: genetic status, pathogenicity, distribution and competitive survival

Introduction

In 1993, a new disease of alder along rivers and horticultural shelterbelts in Britain was diagnosed as a *Phytophthora* disease by R.G. Strouts of the Forestry Commission's Pathology advisory service (Gibbs *et al.*, 1994). It was caused by a *Phytophthora* with a superficial resemblance to *P. cambivora*, a common pathogen of hardwood trees in Europe. However, the alder *Phytophthora* exhibited several unusual properties which suggested it was a species hybrid (Brasier *et al.*,1995). Since then it has been confirmed that the new *Phytophthora* has indeed arisen via a recent interspecific hybridisation event. It is now spreading across Europe as a variety of different hybrid types (Brasier *et al.*, 1999). Some of these types are locally very damaging, posing a serious threat to alder stands and to the stability of riparian ecosystems (see Chapters 4 and 6; Gibbs *et al.*, 1999; Streito and Gibbs, 2000). The alder Phytophthora hybrids may also pose a threat to native alders outside Europe, such as those of North America.

In these circumstances, there is a need to understand the differences between the hybrid types that comprise the alder Phytophthoras, since they may both look and behave like different *Phytophthora* species. The present chapter reviews their genetic status, cultural properties, distribution, pathogenicity and competitive survival.

Hybrid status of the alder Phytophthoras

Initial study of the alder Phytophthora isolates in the UK showed that they differed from *P. cambivora* in being self-fertile rather than self-sterile and outcrossing, in having a submerged rather than an aerial colony type, and in having a lower optimum temperature for growth. They also exhibited an unusually high level of zygotic

abortion. It was this combination of properties that suggested that they might be derived from a species hybrid involving *P. cambivora* as a parent (Brasier *et al.*, 1995). The hybrid hypothesis was investigated in detail by Brasier *et al.* (1999), and they demonstrated that the alder Phytophthora comprised not one, but a range of hereroploid species hybrids. For simplicity, these can be divided into a standard type and several variant types (Table 5.1), as described below.

Standard type

A relatively common, 'standard' alder Phytophthora type occurs across much of Europe, from Scotland and Sweden to Austria and southeast France (Figure 5.1). The basic diploid chromosome number for *Phytophthora*, including *P. cambivora*, is n=10 (Sansome, 1987). The standard type is near-tetraploid (n=18–22) and is unable to complete meiosis beyond metaphase I. Standard isolates have dimorphic sites in the ITS region of their rDNA genes (i.e. they have DNA signatures representative of more than one species), consistent with their being allopolyploids between *P. cambivora* and another *Phytophthora* closely related to *P. fragariae*.

Variant types

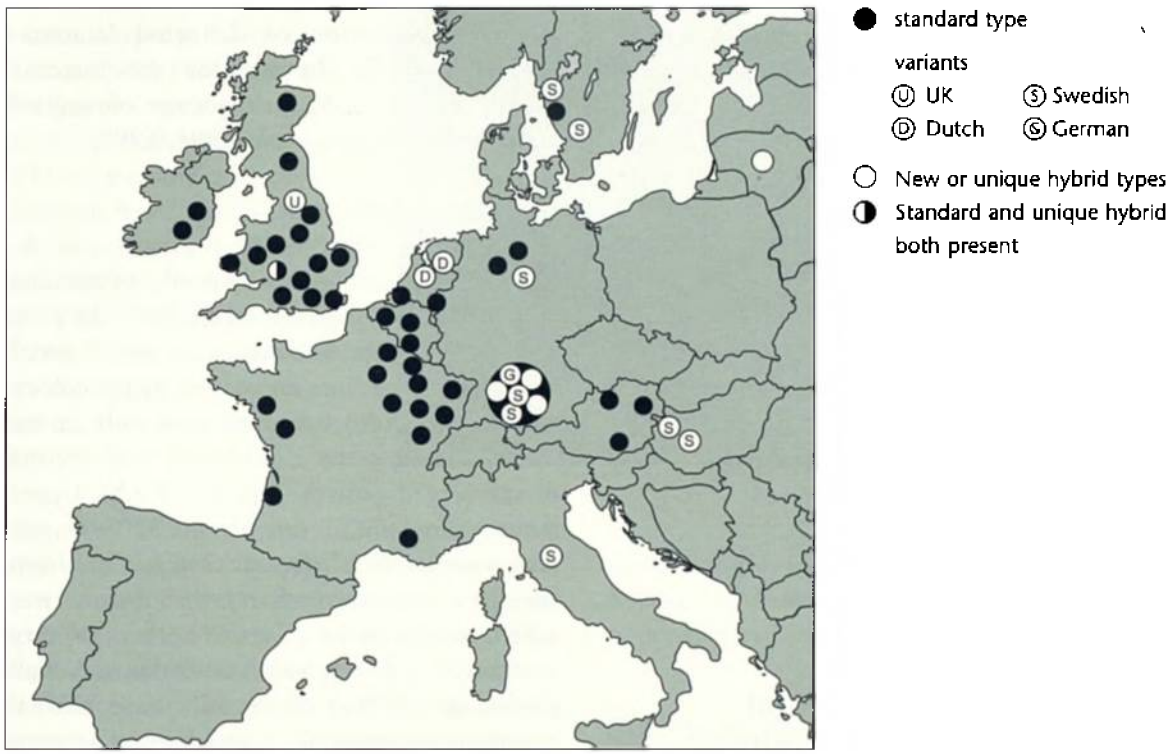
Morphologically, behaviourally and genetically distinct alder Phytophthoras, collectively termed natural variants, also occur across many parts of Europe (Figure 5.1). Up to now, these have been informally labelled according to the country from which they were first

Table 5.1 Comparative cytological and cultural properties of the alder Phytophthoras, *P. cambivora* and *P. fragariae* (from Brasier *et al.*, 1999)

Alder Phytophthora	Chromosome number[a], inferred karyotype	Meiosis	Colony development	Oogonial type[b]	Antheridial type[c]	Optimum/ maximum growth temp. (°C)
Standard type	18–22, ~4*n*±2	Incomplete	Unstable	Ornamented	2A+HA	~25/30
German variant	16–18, ~2*n*+7	Incomplete	Unstable	Ornamented	2A+HA	~27/32
Dutch variant	13–15, ~2*n*+4	Complete	Very unstable	Extremely ornamented	1A+P	~27/32
Swedish variant	11–13, ~2*n*+2	Complete	Very unstable[d]	Smooth	2A	~27/30
UK variant[d]	–	–	–	–	–	–
P. cambivora	10–12, 2*n*	Complete	Stable	Ornamented	2A	~27/34
P. fragariae var. *fragariae* var. *rubi*	10–12, 2*n*	Complete	Stable	Smooth	1A+P	~25/30

[a] A range limit is normally given for *Phytophthora* chromosome numbers.
[b] Smooth walled, see Figure 5.4a; ornamented, Figure 5.4b; extremely ornamented, Figure 5.4c.
[c] 2A, large, two-celled amphigynous antheridia; 2A+HA, predominantly two-celled, some single-celled amphigynous antheridia; 1A+P, predominantly small, single-celled amphigynous plus some paragynous antheridia.
[d] UK variant P841 was too unstable to characterise.

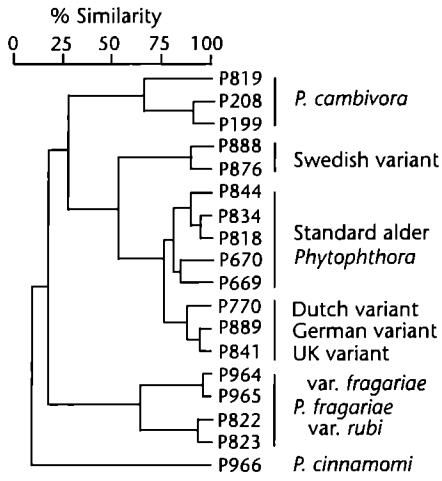
Figure 5.1 Known distribution of alder Phytophthora hybrid types in Europe. Greatly simplified representation based on 280 samples.



obtained, namely as the Dutch, German, Swedish and UK variants. The different variants have different colony morphologies, gametangial types and temperature-growth relationships. They also vary in aggressiveness to alder (page 48). Their chromosome numbers are generally intermediate between diploid and tetraploid (Table 5.1). The lower chromosome number 'Swedish' and 'Dutch' variants are able to complete meiosis. The ITS profiles of the variants differ from those of the standard type, tending either to be more like that of *P. cambivora* or that of *P. fragariae*.

Analysis of AFLP patterns (Figure 5.2) shows that the standard types are evolutionary lineages lying in an intermediate position between *P. cambivora* and *P. fragariae*; while the variants represent further evolutionary lineages lying adjacent to but separate from the standard type (Brasier *et al.*, 1999). The variants might be genetic breakdown products of the standard type, backcross products or products of further hybridisation events. As a whole, the alder Phytophthoras appear to be a swarm of recent species hybrids that are still in process of evolution. Even standard types are apparently still evolving, since they show evidence of continuing recombination in their ITS arrays (Brasier *et al.*, 1999) and in their isozyme patterns (page 46). They also show evidence of continuing change in their gametangial morphology and differ in their aggressiveness. Isozyme and mitrochondrial DNA analyses are in progress with a view to establishing the precise nature of the original hybridisation process. The potential for rapid evolution of plant pathogens via hybridisation is discussed elsewhere (Brasier *et al.*, 1999; Brasier, 2000a, 2001).

Figure 5.2 Dendrogram of relationships between alder Phytophthora hybrid types and the presumptive and putative parent species of the hybrid, *P. cambivora* and *P. fragariae*, based on analysis of AFLP patterns. *P. cinnamomi* is an outgroup. Scale shows genetic distance (from Brasier *et al.*, 1999).



Colony and gametangial characteristics of the alder Phytophthoras

The following are typical cultural characteristics of freshly isolated wild-type standard isolates, and of the different types of variants, growing on carrot agar (CA) at 20°C in darkness.

Standard isolates

Colonies are distinctive, with a flat, appressed felty appearance, either with no aerial mycelium or a little loose woolly aerial growth (Figure 5.3 a–d). Colonies are sometimes irregular, with faster or slower growing sectors. Optimum temperature for growth is *c.* 25°C and upper temperature limit *c.* 29°C (Table 5.1). Gametangia are produced after 4–5 days in darkness. Oogonia are morphologically similar to those of *P. cambivora*, usually varying from near smooth to warty or wavy-verrucose in a single colony, with tapered stalks. Antheridia are mainly large, often two-celled, a proportion one-celled. Small, partially or abnormally developed gametangia are common and characteristic. Often they include a small proportion of distorted 'comma-shaped' oogonia. In addition the oogonia usually exhibit a high frequency of zygotic abortion (see Brasier *et al.*, 1995, 1999).

German variant

The German variant usually produces colonies on CA that do not grow to the edge of the petri dish. It produces a dense, felty-white aerial mycelium, sometimes across the whole colony (cf. *P. cambivora*) but sometimes only in the colony centre; often interspersed with sectors of submerged growth (Figure 5.3 g,h). Upper temperature limit for growth is *c.* 32°C. Gametangia are usually frequent. Oogonia are from smooth to moderately warty, with mainly two-celled, sometimes single-celled antheridia; very rarely with a paragynous antheridium. A high percentage (90%+) of oogonia have normal looking oospores. Colony and fertility patterns can vary widely from one growth test to the next.

Dutch variant

The colonies of the Dutch variant tend to be irregular, sometimes mainly submerged and sometimes with dense white aerial mycelium, often failing to grow to the edge of the petri dish (Figure 5.3 i,j). Upper temperature limit for growth is *c.* 32°C. Usually, more gametangia tend to be formed at the edge than in the centre of the colony. Oogonia are often extremely ornamented with unusual coraloid outgrowths (Figure 5.4 c) and many are highly distorted. Within one colony, most oogonia have one-celled amphigynous antheridia but some may have two-celled antheridia and a small proportion may have single-celled, paragynous antheridia.

UK variant (P841)

The UK variant often exhibits an irregular

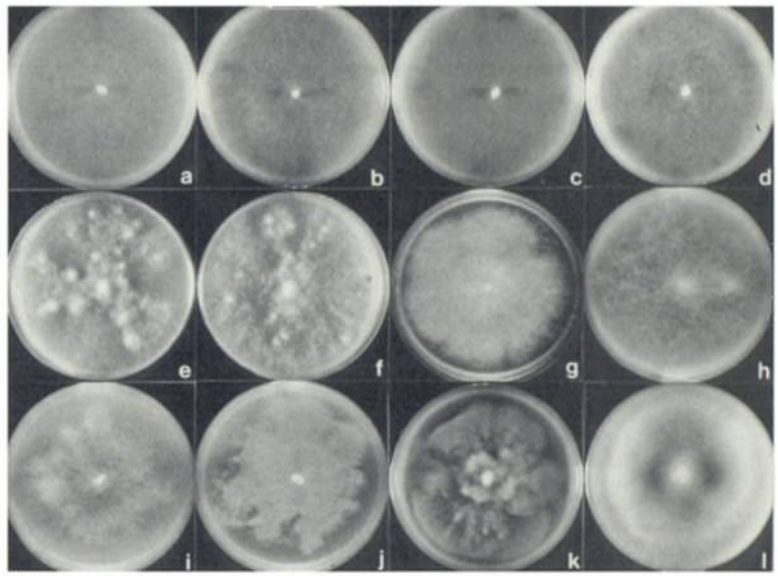


Figure 5.3 Colony types of the alder Phytophthoras:
a–d: Standard isolates from UK, France, Austria and Germany (Isolates P671, P834, P844 and P818 respectively). **e,f**: Swedish natural variant: **e**: common type (P1265); **f**: colony exhibiting chimaeric fertile patches (P876). **g,h**: German natural variant (P889 and P890). **i, j**: Dutch variant (P770 and P972). **k**: UK variant (P841). **l**: Cultural variant (P818v) which originated as a sector of standard isolate P818 (see **d**).

striate colony with sexually sterile sectors interspersed with entirely submerged sexually fertile sectors (Figure 5.3 k). However, this variant is characteristically variable from one subculture to the next. The combinations of colony patterns, growth rates and temperature-growth limits, fertility to sterility and gametangial morphologies observed are remarkable (see Brasier *et al.*, 1999; Delcan and Brasier, 2001). The variation in gametangial types among single hyphal tip cultures is shown in Figure 5.5.

Swedish variant

The Swedish variant has an irregular appressed colony, often with woolly aerial mycelium in the colony centre but submerged growth at the edge (Figure 5.3 e). Gametangia are frequent. Upper temperature limit for growth is *c.* 29°C. Some cultures are highly unstable and chimaeric (Figure 5.3 f), with gametangia produced only beneath patches of aerial mycelium. Oogonia are mostly smooth-walled, but some are slightly wavy edged-verrucose. Occasionally, oogonia have large, distorted beak-like protuberances. Antheridia are consistently two-celled and amphigynous (the antheridial cross-wall is often close to the base of the antheridium, and can therefore be overlooked). A high percentage (90%+) of oogonia have normal looking oospores.

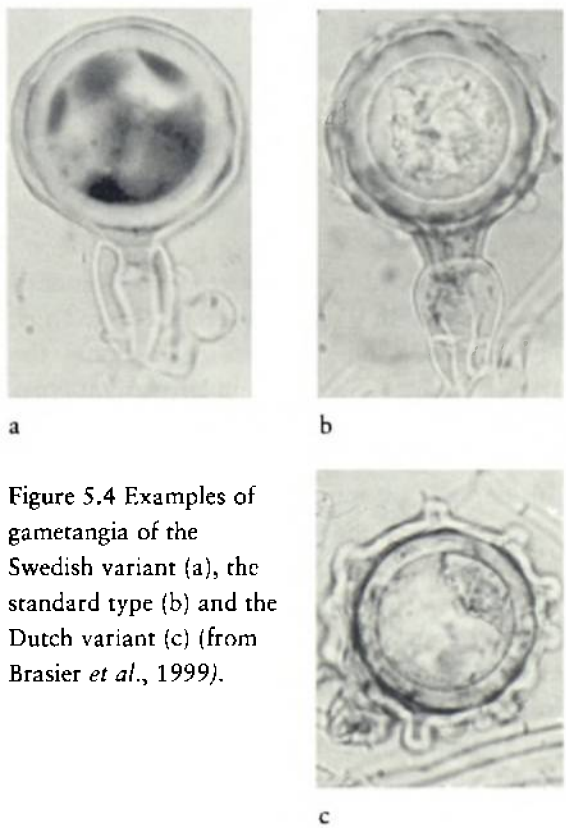
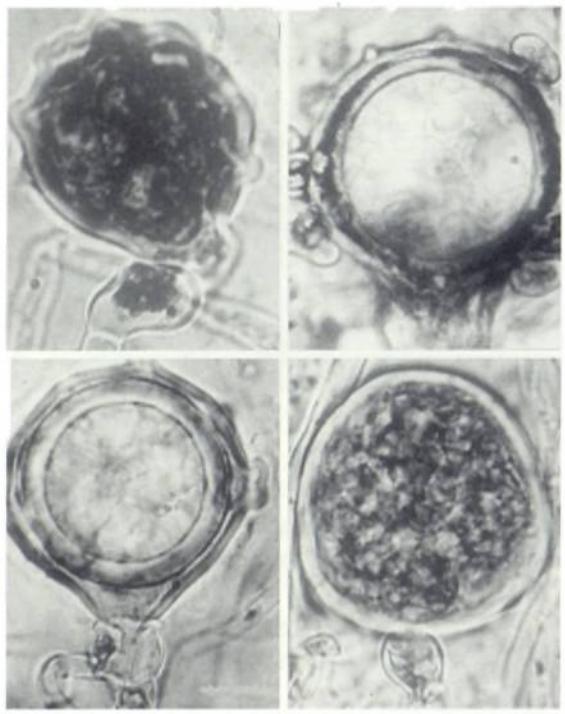


Figure 5.4 Examples of gametangia of the Swedish variant (a), the standard type (b) and the Dutch variant (c) (from Brasier *et al.*, 1999).

Figure 5.5 Different gametangial types among four single hyphal tip colonies taken from the same colony plate of the UK variant (P841). From J. Delcan and C. M. Brasier, previously unpublished.



Instability of the standard hybrid in storage

It has proved difficult to keep the standard alder Phytophthora in long-term storage. Thus, in one study, nine isolates representing the Dutch, German, Swedish and UK variant types remained comparatively stable during storage under paraffin oil for up to 4 years. However, 30 out of 58 standard alder Phytophthora isolates stored under similar conditions were found to be no longer wild-type when re-subcultured (Delcan and Brasier, 2001). They exhibited markedly altered colony patterns and changes in fecundity levels, including complete loss of gametangial production. This tendency can make it difficult to decide whether some cultures received from other culture collections have changed during storage or represent novel (non-standard) phenotypes. For this reason, it is very important that cultural properties of an alder Phytophthora isolate should be recorded when fresh, i.e. at time of isolation.

Genetic stability, oospore viability and origin of the variants

An actively growing colony of one standard isolate, P818 (Figure 5.3 d), gave rise to a sector with a different colony type, a different gametangial type and a 'new' ITS profile that was identical to the ITS type of the Dutch, German and UK variants). Sector of P818 therefore resembled a natural variant (Delcan and Brasier, 2001). This suggested that the natural variants might arise via sexual or somatic segregation in the standard hybrid.

Delcan and Brasier (2001) have investigated this hypothesis. Oospore viability, oospore germinability and phenotypic variation among zoospore and hyphal tip derivatives of seven standard or natural variant isolates were examined. *P. cambivora* was included as a 'parent' species. Oospore viability in the standard hybrid, estimated by the tetrazolium bromide method, was low (*c.* 31–36%). No germination was observed in >4000 oospores tested, although germination did occur in the *P. cactorum*, *P. citricola* and *P. cambivora* controls. This is consistent with the known meiotic irregularities in the standard hybrid. Mean oospore viabilities in the natural variants were significantly different ($P < 0.001$), ranging from *c.* 24 % in the UK variant to *c.* 75 % in the Dutch variant. Again, no oospore germination was observed.

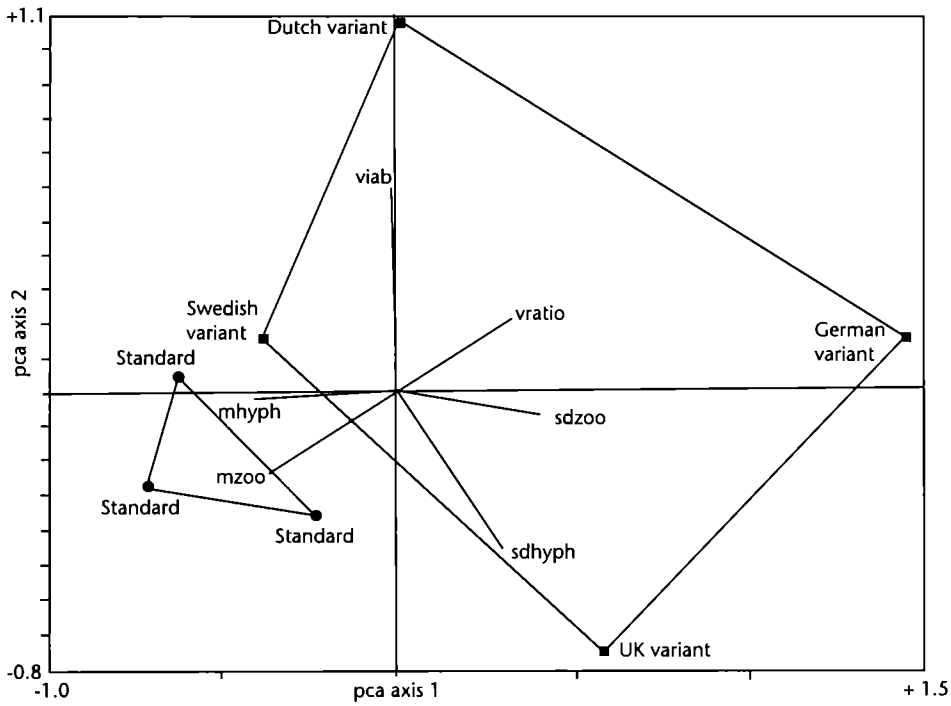
In the same study, colonies grown from zoospore and hyphal tip derivatives of the standard hybrid and of the Swedish and Dutch variants resembled the 'parent' isolate in phenotype. The derivatives of the German and UK variants, however, often differed from the parent type. Those of the UK variant were extremely and continuously variable in colony

patterns, growth rates, temperature–growth relationships and fertility levels.

These results did not, therefore, support the hypothesis that the natural variants arise as segregants or genetic breakdown products of the standard hybrid. However, this possibility cannot yet be excluded, especially taking into consideration the sector obtained from isolate P818v, and the considerable potential for genomic reorganisation that must exist in an allopolyploid species hybrid. An alternative explanation is that the natural variants arise via backcrosses and intercrosses between standard hybrids and the parent species.

A biplot of a Principal Component Analysis summarising the relationships between the alder Phytophthoras examined in the above study is shown in Figure 5.6. Some 80% of the overall variation was accounted for by the two axes in the biplot. Three standard hybrid isolates grouped closely, indicating that they behaved similarly with regard to these variables. An isolate of the Swedish variant also aligned close to the standard isolates, indicating a comparable pattern of behaviour. Those of the UK, German and Dutch variants, however, were widely separated, indicating that each exhibited a relatively unique and inconsistent set of variables.

Figure 5.6 Biplot of a principal component analysis based on the phenotypic behaviour of colonies of standard and variant types of the alder Phytophthora and their asexual derivatives. Note the similarity in response of the standard isolates examined and the wide differences between the UK, Dutch and German variants. Vectors are as follows: sdhyph: SD vector of growth rates of hyphal tip derivatives with respect to the parent isolate; sdzoo: SD vector of growth rate of zoospore derivatives with respect to the parent isolate; mhyph: hyphal tip growth rate vector with respect to the parent; mzoo: zoospore growth rate vector with respect to the parent; viab: oospore viability; vratio: ratio of replicate/theoretical SE of oospore viability. From Delcan and Brasier (2001).



Isozyme characterisation of hybrid types

Olsson (1999) successfully used isozymes to discriminate the Swedish variant from the standard hybrid. Recently, further isozyme studies using six enzymes (W. Man In't Veldt and C.M. Brasier, unpublished) have shown that the standard hybrids exhibit complex banding patterns characteristic of an allotetraploid. These studies also indicate that changes from heterozygosity to homozygosity are occurring in standard isolates, consistent with continuing evolution of their hybrid genome. The Dutch and German and UK variants share a unique and characteristic isozyme pattern. The Swedish variant exhibited another unique pattern. The isozyme data studies are therefore consistent with the AFLP data (Figure 5.2). They also support the view that *P. cambivora* is one of the parents of the hybrid, whereas they indicate that *P. fragariae sensu stricto* is unlikely to be a direct parent.

Distribution of the hybrid types in Europe

Since 1993 over 280 samples of the alder Phytopthoras received by the Forestry Commission have been assigned to a hybrid type. Most isolates have fallen into the above previously identified hybrid categories, but a number have also been identified as new variants (i.e. as previously unknown, unique hybrid types). With standard isolates the assignment has been made mainly on the basis of colony characteristics, inability to grow at 30°C, gametangial morphology and a high zygotic abortion level. A proportion of standard isolates are routinely checked with isozymes and, more recently, with a RAPD protocol (C. M. Brasier and S. A. Kirk, unpublished). Assignment of natural variants has usually included investigation of additional properties such as chromosome number, ITS profile and AFLPs of genomic DNA. Valuable collaboration in this work has been provided by D.E.L. Cooke and J. M. Duncan of the Scottish Crops Research Station and W. A. Man in't Veld of the Netherlands Plant Protection Service.

The present known distribution of different hybrid types in Europe is summarised in greatly simplified form in Figure 5.1. The standard hybrid type is fairly widely distributed across Britain, Ireland, France, Belgium, Germany and Austria. It is also present in southern Holland (Limburg Province). The Swedish variant is now known from Sweden, north and south Germany, Hungary and Italy. This may represent the spread of this genotype by the international nursery trade (see also Gibbs *et al.*, Chapter 6). The Dutch, German and UK variants are so far known only from their 'namesake' countries.

Over 120 isolates have been examined from Bavaria, southern Germany. Here, several previously unknown hybrid types (new variants) have recently been found alongside the standard hybrid and the German and Swedish variants (C.M. Brasier, S.A. Kirk and T. Jung, unpublished). Overall, variant types make up *c.* 20% of the Bavarian population and standard types *c.* 80%. Bavaria may therefore represent a hot spot of evolutionary activity. Previously unknown hybrid types are also present at one site in western Britain (Hadley Brook, Worcestershire) and in Lithuania (Figure 5.1).

Comparative aggressiveness of alder Phytophthoras

The alder Phytophthoras can cause a rapid necrosis of the inner bark of the collar and stems of alder trees in the field (Streito, Chapter 4; Lonsdale, Chapter 7). Sometimes lesions may extend over two metres above soil level. It is therefore important to understand

the potential aggressiveness of the different hybrid types.

Preliminary observations on aggressiveness

In June 1994, several young *A. glutinosa* trees were wound inoculated in the field, using inoculum plugs taken from cultures of standard isolates (Brasier *et al.*, 1995; Gibbs, 1995). Over 3 months, the mean extension rate of lesions was *c.* 1.5 mm day^{-1}. In another study, 15-month old seedlings of *A. glutinosa* were wound inoculated with three standard hybrid isolates (Gibbs, Chapter 8). The seedlings were inoculated on the stem *c.* 7 cm above soil level and after 15 days there were significant differences in the overall mean daily extension rates of the three isolates (Table 5.2).

Inoculation of large excised alder stems

A simple linear method was needed for assessing the comparative aggressiveness of large numbers of alder Phytophthora isolates. The elm log wound inoculation method of Webber and Hedger (1986) was therefore adapted to alder. Between April 1995 and November 1998, 12 separate experiments were conducted using excised logs of *A. glutinosa*. The logs were incubated at 20°C for 5–6 weeks, with 8–10 replicates for each isolate. In total 19 geographically representative standard alder Phytophthora isolates and 9 isolates representing the UK, Dutch, German and Swedish variant types were tested. Also tested were: isolate P818v (the morphologically unique colony sector of standard isolate P818), 11 isolates of *P. cambivora*, an isolate of *P. fragariae* var. *rubi*, 21 isolates representing *P. cinnamomi* (a common tree pathogen in Europe), *P. gonapodyides*, *Phytophthora* sp. 'O Group' (Brasier *et al.*, 1993a), *P. citricola* and *P. megasperma*. The latter are four *Phytophthora* morphospecies found in the same riparian ecosystems as the alder Phytophthoras. Certain alder isolates (e.g. standard isolate P772 and Dutch variant P770) were used repeatedly to maintain a level of continuity between experiments. All material was destroyed at the conclusion of each study.

Detailed results of these experiments are presented in Brasier and Kirk (2001). In summary, most isolates of the standard hybrid isolate and those of the Dutch variant type were highly aggressive to alder bark. Generally they produced lesions of *c.* 300 cm^2 (common range *c.*100–500 cm^2) after 5 weeks (Table. 5.4). The isolates of the 'Swedish', 'UK' and 'German variants', and of *P. cambivora*, were only weakly pathogenic. P818v resembled P818 in its pathogenicity.

Table 5.2 Inoculation of standard alder *Phytophthora* isolates into *A.glutinosa* seedlings[a]

Isolate code	Origin	Number of seedlings	Mean lesion extension (mm day^{-1})
P766	River Windrush, Oxfordshire	70	2.2a[b]
P772	River Don, Yorkshire	70	2.7ab
P807	River Rother, Hampshire	70	3.1b

[a]For details of experimental procedures see Gibbs (Chapter 8).

[b]Figures followed by the same letter do not differ statistically from each other.

The results of one experiment, Experiment XII, are shown in Table 5.3. In this test, the mean lesion area of the three standard isolates was 99.1 cm^2; that of two Dutch variant isolates 50.4 cm^2; that of the five German, UK and Swedish variant isolates combined 15.8 cm^2; and that of the two *P. cambivora* isolates 7.4 cm^2. The means of the standard isolate and Dutch variant groups were significantly different from each other, from the means of the combined German, UK and Swedish variant group, and from *P. cambivora* ($P< 0.05$). The mean of the combined German, UK and Swedish variant group, however, was not significantly different from that of *P. cambivora*. Overall an approximate order of pathogenicity was: standard isolates > Dutch variant > German variant > Swedish variant, UK variant and *P. cambivora*. In addition, the tests showed isolates of *P. fragariae*, *P. cinnamomi*, *P.* sp. 'O-group', *P. cryptogea*, *P. megasperma*, *P. gonapodyides* and *P. citricola* to be only very weakly pathogenic or non-pathogenic to alder bark.

Although the genetic mechanism underlying the origin of the variant types (e.g. via breakdown products of the standard type, or via backcrosses) remains unclear, the Swedish, Dutch and UK variants appear to have lost some pathogenicity to alder in the process. The results suggest therefore, that the disease is likely to be more damaging in regions where the standard type predominates, such as the UK, France, Belgium and Austria. Nonetheless,

Table 5.3 Lesion areas of alder Phythophthora isolates and *P. cambivora* isolates on alder bark in Experiment XII

Isolate number	Isolate type or species	Mean lesion area (cm^2) and SE[a]	
P938	Standard type	108.0 ± 11.9	a
P834	Standard type	102.6 ± 8.5	a
P772	Standard type	86.6 ± 13.1	a, b
P972	Dutch variant	75.7 ± 11.7	b
P890	German variant	28.9 ± 3.7	c
P770	Dutch variant	25.1 ± 4.5	c
P889	German variant	22.3 ± 6.4	c, d
P841	UK variant	11.9 ± 2.2	d, e
P819	*P. cambivora*	10.8 ± 3.4	d, e
P887	Swedish variant	9.7 ± 1.3	d, e
P888	Swedish variant	6.3 ± 1.2	e
P821	*P. cambivora*	4.0 ± 0.9	e
Control		1.7 ± 0.1	e
Isolate groups[b]			
Standard isolates (3)		99.1 ± 6.4	x
Dutch variant (2)		50.4 ± 25.3	y
German+Swedish+UK variant (5)		15.8 ± 4.2	z
P. cambivora		7.4 ± 3.4	z

[a]Lesion areas followed by a different letter are significantly different at the 95% level ($P<0.05$).

[b]Number of isolates in parentheses.

Table 5.4 Seasonal influence on lesion sizes produced by standard alder Phytophthora isolates[a]

Experiment number	Date of inoculation	Number of isolates tested	Mean longitudinal lesion extension rate (mm day^{-1}) and SE	Mean lesion area (cm^2) ± SE	Largest mean lesion area of any isolate
XI	14.07.98	5	5.8 ± 0.2	354 ± 32	415.6
VII	08.10.96	4	5.5 ± 0.3	408 ± 55	517.5
XII	21.10.98	3	5.0 ± 0.1	99 ± 6	108.0
VIII	22.10.96	4	4.3 ± 0.3	240 ± 56	353.4
IV	12.02.96	3	3.2 ± 0.7	100 ± 35	139.6
II	04.10.95	3	3.1 ± 1.2	225 ± 107	385.7
IX	19.12.96	1	2.0	26	26.0
III	13.12.95	8	1.6 ± 0.4	26 ± 8	71.0
X	02.02.98	3	1.9 ± 0.1	25 ± 2	27.3
V	04.03.96	10	0.8 ± 0.2	12 ± 4	36.7
I	17.04.95	4	nil/trace	nil/trace	(1.0) nil/trace
VI	15.04.96	4	nil/trace	nil/trace	(1.0) nil/trace

[a]Experiments are shown in descending rank according to mean longitudinal extension rate.

all the natural variants were originally isolated either from alder bark or from a root lesion on alder and must therefore have some ability to attack alder in the field.

Seasonal differences in susceptibility and critical threshold effects

Over the 12 experiments, lesion development varied with the season (Table 5.4). Lesions were largest on alder logs cut during July–October, average linear extension rate ranging from 3.1–5.8 mm day^{-1}; they were smaller on logs cut between November and March, with extension rates of 0.8–3.2 mm day^{-1}, while lesions failed to develop at all on logs cut during April. Also in some experiments, the standard isolates separated into groups of 'higher' and 'lower' levels of aggressiveness. The relative position (high or low) of control standard isolate P772 in relation to the other standard isolates also varied. It should be emphasised that the log-inoculation method tests the ability of an isolate to spread in living host tissue, but not the capacity for infection and establishment in the host. Nonetheless, these results indicate that season influences host susceptibility and that critical thresholds of host resistance were operating in these experiments. In the field, such threshold effects could mean the difference between chronic, suppressed disease and acute, potentially lethal disease.

Host range: pathogenicity to bark of other tree species

P. cambivora, a confirmed parent of the hybrid alder Phytophthoras, attacks bark of a broad range of tree species in Europe (Brasier, 2000). There is therefore a concern that the hybrids might be able to affect other tree species (Brasier *et al.*, 1999). In consequence, tests of their potential host range have been carried out in the laboratory and in the field.

Inoculation of riparian trees

In May 1997 a wound inoculation experiment was carried out on small (3–9 cm dbh) saplings of *A. glutinosa*, *Acer pseudoplatanus*, *Betula pendula*, *Coryllus avellana*, *Crataegus monogyna*, *Fraxinus excelsior*, *Salix caprea*, *S. fragilis*, *S. purpurea* and *Ulmus glabra* (J.N. Gibbs, personal communication). *Betula* is an alder relative, while *Salix* is another important riparian genus. The trees were located in a plot close to a disease infested river in south Wales. A local standard alder *Phytophthora* isolate, P670 (see Brasier *et al.*, 1995 and Brasier and Kirk, 2001), was used in the tests. Wound inoculations were made 10–40 cm above ground level with five replicates for each tree species. After 6 weeks small lesions (mean extension rate *c.*1 mm day^{-1}) had developed on all the inoculated alders (N.B. the relatively small size of these lesions may reflect the season of inoculation, see Table 5.4). No lesions were produced on any of the other tree species.

Inoculation of excised stems of *Quercus*, *Fagus*, and other *Castanea* tree species

Controlled tests were conducted at 20°C using the log inoculation procedure described above (cf. Table 5.4). Tree species tested were the broadleaves *Castanea sativa*, *Quercus robur*, *Fagus sylvatica*, *Acer pennsylvannicum* and the conifers *Chamaecyparis lawsoniana* and *Taxus baccata*. Five isolates of the standard hybrid and an isolate of the Dutch variant P770 were used. All were non-pathogenic to the bark of these trees but produced substantial lesions on *Alnus* in the same experiments. In contrast, *P. cambivora* was an aggressive pathogen on the logs of *Quercus* and *Castanea* and a significant pathogen on some other species. *P. cinnamomi* and *P. citricola* also produced substantial lesions on several of the other hosts (Brasier and Kirk, 2001).

These studies indicate that the alder Phytophthoras are relatively specific to alder (Brasier and Kirk, 2001). This in turn suggests that a novel host specificity – specificity to alder – may have been acquired as a result of the hybridisation process, providing them with a fitness advantage over the parents (Brasier *et al.*, 1999; see also Ersek *et al.*, 1995).

Competitive survival of the alder Phytophthoras

The alder Phytophthoras exhibit some unusual properties, including their tendency to genetic instability, the failure of their oospores to germinate, differences in temperature–growth relations and their pathogenicity. These properties raise questions about their general survival ability (as spores or mycelium) in competition with other *Phytophthora* species commonly present in riparian ecosystems, such as *P. gonapodyides* (Brasier *et al.*, 1993; Brasier and Kirk, 2001).

Survival in bark and soil

Limited field sampling and experimental work has been carried out to investigate their competitive survival. During the summer of 1997 a number of river systems in southern Britain, in areas with the standard hybrid, were sampled for *Phytophthora* species (J. Delcan and C. M. Brasier, unpublished). Sampling was carried out simultaneously from bark necroses on diseased alder trees and from soil around collars of affected trees (Table 5.5). A site with disease in an alder shelterbelt around a strawberry field was also sampled (Table 5.6). The sampling methods involved inserting soil or necrotic bark into apple baits (Brasier and Strouts, 1975) and baiting of flooded soil or river water with young hydromorphic alder or Eucalyptus leaves (cf. the oak leaf isolation method of Jung *et al.*,1996).

The standard alder Phytophthora was isolated consistently from necrotic lesions on the affected trees (Tables 5.5 and 5.6), but it was isolated only once from soil around the

Table 5.5 Frequency of *Phytophthora* taxa isolated from bark necroses on alder trees and soil around affected trees: May–July 1997 (J. Delcan and C.M. Brasier, unpublished).

Site	Bark necrosis		Soil around base of affected trees				
	Standard alder Phytoph-thora	Other Phytoph-thora or Pythiums	Standard alder Phytoph-thora	*P. gona-podyides*	*P. citricola*	*P. mega-sperma*	*Pythium* spp.
River Wey, Surrey	2	0	0	27	0	1	1
River Rother, Hampshire	4	0	0	10	2	0	51
River Rother, Sussex	4	0	0	43	0	0	16
River Usk, Powys	2	0	0	13	0	0	13
River Clun, Shropshire	1	0	0	0	4	0	8
River Wye, Worcestershire	8	0	0	8	0	0	26
Totals	**21**	**0**	**0**	**101**	**6**	**1**	**0**

Table 5.6 Frequency of *Phytophthora* taxa in bark necroses on alder trees and soil around affected trees: *Alnus incana* shelterbelt around strawberry field, Hampshire, UK, May 1997 (J. Delcan and C. M. Brasier, unpublished).

Location	Standard alder Phytophthora	*P. citricola*	*P. cactorum*	*P. megasperma*	*Pythium* spp.
Bark necrosis[a]	0	0	0	0	0
Soil close to affected trees	1	21	3	0	7
Soil 5 m from affected trees	0	3	0	1	15

[a]Several trees had alder bark lesions but only one had fresh lesions suitable for isolation.

stem base of an affected tree. In contrast, several *Phytophthora* species: *P. gonapodyides*, *P. citricola*, *P. megasperma sensu stricto*, and a previously unknown riparian *Phytophthora* species related to *P. gonapodyides* (known as '*P.* taxon Riversoil'), were locally abundant or occasional in soil around the diseased alder trees. They were never isolated from the bark lesions on the trees. Similarly in Bavaria, standard alder *Phytopthora* was readily isolated from bark of alders growing in a severely diseased plantation, but baiting of the rhizosphere spoil yielded only *P. citricola* and *P. cactorum* (T. Jung, personal communication).

Occurrence in water

During 1998 and 1999, J-C. Streito (personal communication) attempted isolation of Phytophthoras directly from river water at three sites in northeast France locally infested with the standard alder Phytophthora (see also Streito *et al.*, 2002 and Streito, Chapter 4). Freshly cut alder twig rafts were floated on the water as baits. Direct isolations were made from multiple fragments taken from the exposed cut ends of each twig. Overall, the number of positive isolations of the standard alder Phytophthora was very low: >1% of fragments of necrotic bark yielded the alder Phytophthora. However, as in the soil tests (above), the raft fragments frequently yielded other Phytophthoras, especially *P. gonopodyides*. Standard alder *Phytophthora* isolates were readily isolated from bark lesions on alders at the same sites.

Intriguingly, in one raft sampled at Liverdun in July 1998, 11 of the 12 twig-ends yielded the alder Phytophthora. This is in contrast to the results of the other 15 rafts sampled at this site during 1998. Among these, mostly 0 but occasionally 1–3 of the 12 twig ends were positive for the alder Phytophthora. An even greater contrast is provided by data from the same site during 1999 when another 54 rafts were sampled: all these were negative. This suggests that an unusually intense spike of zoospore activity ocurred in the water at the Liverdun site in July 1998.

Therefore, it has proved difficult to isolate the standard alder Phytophthora both from soil around the base of diseased alder trees in the field and from river water. Furthermore, in a preliminary experiment on survival of the standard alder Phytophthora, in which mycelium and oospores were incorporated into unsterile riverbank soil in pots, the alder Phytophthora could not be reisolated from the soil after only 1 month's incubation at 20°C. Yet both *P. gonapodyides* and '*P.* taxon Riversoil' were isolated from the same soil samples after 2 months (Delcan and Brasier, 2001). In another test, J-C. Streito (personal communication) buried pieces of inner bark of alder inoculated with standard alder *Phytophthora* in unsterile soil for 3 months. In this case, the fungus was successfully recovered from the bark pieces, indicating that the alder Phytophthora did survive in the alder debris.

These observations are preliminary and need to be repeated. Nevertheless, along with the low oospore viability of the alder Phytophthoras, they suggest that:

- Oospores are unlikely to contribute significantly to survival and spread of the standard hybrid in nature. (Survival in the absence of oospores is not unusual for a *Phytophthora*. Other root-infecting species, including *P. gonapodyides* and *P. cambivora*, appear to accomplish their lifecycles successfully without regular production of oospores.)
- The standard alder Phytophthora has relatively poor survival ability in soil, especially compared to the common, established oomycete inhabitants of riparian ecosystems such as *P. gonapodyides, P. citricola* and *Pythium*. Possibly, propagules of the standard alder *Phytophthora* are quickly replaced in soil by other microorganisms.
- Mycelium or other propagules of the standard hybrid may successfully perennate in bark debris.
- Release of zoospore inoculum along infested rivers is rare but there may be synchronised spikes of intense activity.
- Zoospores of *P. gonapodyides*, which tend to be extremely abundant in riverwater (C. M. Brasier and E. M. Hansen, unpublished), may numerically or even antagonistically outcompete zoospores of the alder Phytopthoras in the infection court.

In the light of these observations, local spread of the standard alder Phytophthora

along river systems may be mainly via dispersal of infected alder bark or root debris containing perennating mycelium. There may also be spikes of zoospore activity. In either case, it is likely that competition may occur from other *Phytophthoras* in the environment, such as *P. gonapodyides*. Spread over longer distances, including international spread, may be mainly via distribution and planting of infested nursery stock. This is indicated by the geographical distribution of the Swedish variant (Figure 5.1).

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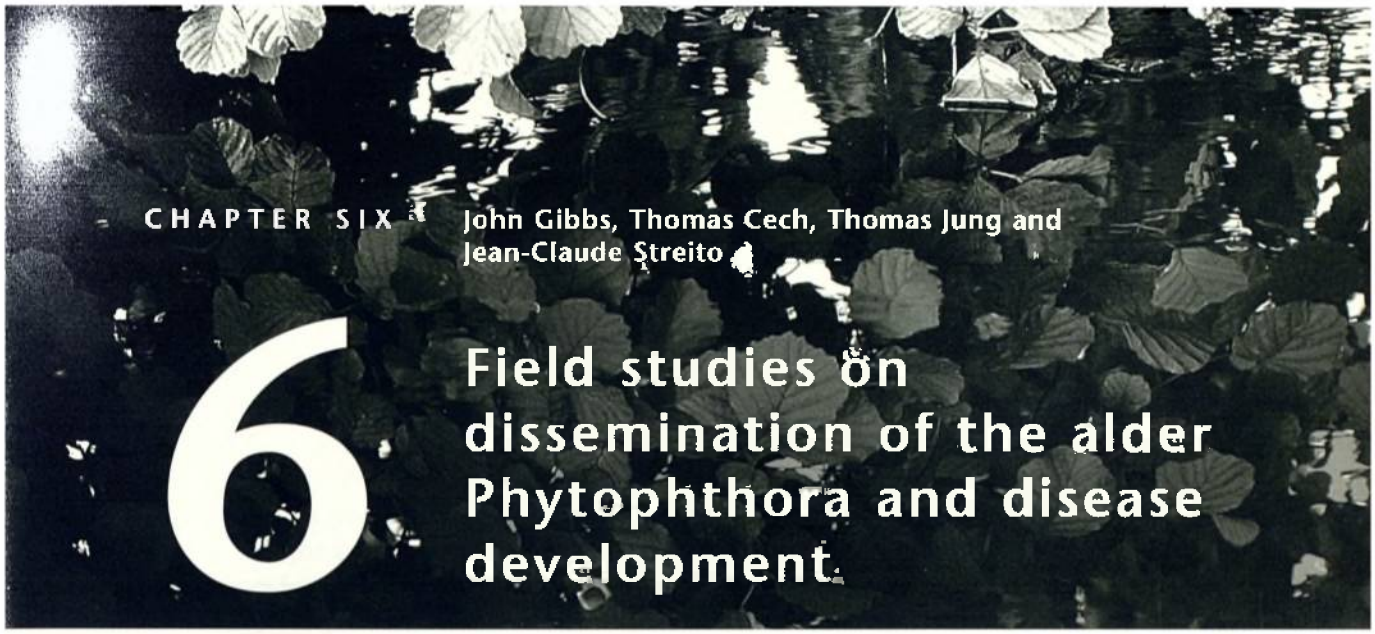


CHAPTER SIX

John Gibbs, Thomas Cech, Thomas Jung and Jean-Claude Streito

6 Field studies on dissemination of the alder Phytophthora and disease development

Introduction

An account of the history and distribution of Phytophthora disease of alder in Europe has been provided by Streito (Chapter 4). This chapter is concerned with the way in which studies on particular sites have informed our understanding of pathogen dissemination and disease development. It supplements the more detailed observations on the relationship between the pathogen and the host described by Lonsdale (Chapter 7).

The chapter is divided into two sections. The first deals with the disease as it occurs in relation to trees in riparian habitats – where trees grow on the banks of rivers and other bodies of water. A link between the disease and watercourses was suspected as soon as the preliminary UK surveys in September 1993 showed that trees in a broadly similar state of disease could be found along lengthy tracts of affected streams (Gibbs, 1995). During the last few years, studies have been initiated on many riparian sites and this work is reported here. Datasets for disease development in three countries are summarised.

The second section discusses the occurrence of the disease in non-riparian sites. That the disease could occur in non-riparian sites was also recognised back in 1993, when the pathogen was isolated from symptomatic trees in a young woodland plantation in east Wales, UK (Gibbs, 1995). Studies on this site quickly lead on to an investigation of planting stock as a source of infection and this work, now extended to a number of European countries, is described here. In addition, data from studies of disease in other non-riparian sites such as orchard shelterbelts are presented.

The disease on riparian sites

Water, or the lack of it, plays a key part in the development of most plant diseases. Its role is likely to be absolutely crucial when the pathogen is a zoospore-producing *Phytophthora* and the host is a tree that often lives with its roots in a river. Water can be expected to play a part in disseminating pathogen, determining the suitability of conditions at the infection court and, through influencing host vigour, affect the pathogen's ability to invade the tissues.

Dissemination of the pathogen by water

Field observations on the distribution of disease in relation to watercourses indicate that water brings the pathogen to the host. This can happen in the slow moving water of a canal or in the fast moving water of a river in spate. The disease can sometimes be found in trees which only experience rare and very transient exposure to flood water. For example, on the River Dee in northeast Scotland, the disease has appeared in trees growing at the 'high-water mark' on banks 3–4 m above 'normal' river height (Figure 6.1).

In the process of dissemination, water must also dilute the number of propagules available for infection – particularly where trees are growing at the edge of lakes or other large bodies of water. At times of transient flooding the dilution of pathogen inoculum must be enormous, but infection can apparently still take place. At one site in Cumbria, northern England, the disease occurred in July 1999 in three small alders on the edge of a small pond 200 m away from the River Leven. This pond, set in a wooded nature reserve, is only flooded on the rare occasions when large quantities of flood water coming down the river are held back by very high tides in the adjacent Morecambe Bay but, nonetheless, sufficient inoculum for infection evidently reached the trees.

Water and the infection court

As yet, there has been little detailed study of the infection court (see Lonsdale, Chapter 7) but there is a variety of field evidence that can be taken into account. Infection of riparian trees can occur without the stem base being flooded – as is witnessed by disease in canal-side trees already mentioned. On such trees the

Figure 6.1 Phytophthora disease in *A. glutinosa* on a bank *c.* 3 m above the Dee, a river in northeast Scotland subject to transient 'spates'. Debris was present at the base of the affected trees showing that only the highest floods reach this point (J.N. Gibbs).

roots are submerged in the water and hence potentially in contact with any fungal propagules that may be present, but rigid control of the water level means that the stem base remains dry. In general, however, inundation of the stem base seems to be favourable for the establishment of disease. One situation, which appears to be particularly conducive to serious disease, arises when alders are growing in low-lying ground from which flood water is slow to drain away. A striking example of this was provided in the UK at an experimental planting of *A. glutinosa* established in 1996 in a water meadow beside the River Rother (see Gibbs, Chapter 8). In 1999 many young trees developed basal stem lesions and it was found that disease incidence was 56% on that part of the experiment where 10–20 cm of water remained for some days after flooding and only 11% on the remainder of the site, which quickly became dry.

Where there is transient flooding of the stem base, the accumulation of flood debris around, or even in, the tree may be important for disease development. On the River Dee in Scotland, a small tree was found to have an 'aerial' bark lesion about 50 cm from ground level which was centred on an accumulation of flood-transported material. It is possible that the pathogen might be present in such debris and also that the debris itself might also be important in creating conditions conducive to infection, perhaps by maintaining a humid environment at the stem surface.

Influence of water on the vulnerability of the host to disease

In some localities, observations have suggested that raised water levels, especially artificially raised levels, could increase the incidence of disease. Thus, as described in Streito (Chapter 4), C. Van Dijk and H. de Gruyter noted that the serious disease that was discovered in 2000 on two small streams in the south of the Netherlands followed the artificial raising of the water level in these streams some time earlier. In Austria T. Cech (Records of the CA) observed that the only occurrence of disease in the white-water rivers of the Tyrol was at a location on the River Inn where water levels had been raised following the construction of a power station.

Quantitative disease data on riparian sites subject to flooding

One of the objectives of the Concerted Action was to encourage Partners to acquire quantitative information on the rate of disease development on various sites. Several years of data are now available for three countries: the UK, France and Austria.

The United Kingdom

In 1994 a survey was established on a grid basis across southern England and east Wales and this has continued with some modification until the present. The structure of the survey is described in detail in Gibbs *et al.* (1999) but, in essence, 100 m plots were established on rivers over 8 m wide and details of alders growing within 10 m of the water's edge recorded. Two principal means of presenting the data have been devised (Table 6.1). One is the percentage of trees that shows classic crown symptoms of the disease (see Streito, Chapter 4) or is dead. This figure has increased from 4.3% in 1994 to 12.8% in 2000. The other measure is the 'Annual Incidence of Disease' (AID). This is based on the numbers of trees becoming diseased or dying in any one year, and takes account of trees that have been killed by the disease but have disappeared, usually by being washed away. The year 1996 was bad, with an AID of 2.51 in contrast to a figure of only 1.09 the previous year. At the time is seemed possible that losses might double each year but in the event the AID for both 1997 and 1998 was much lower. It increased again in 1999 and

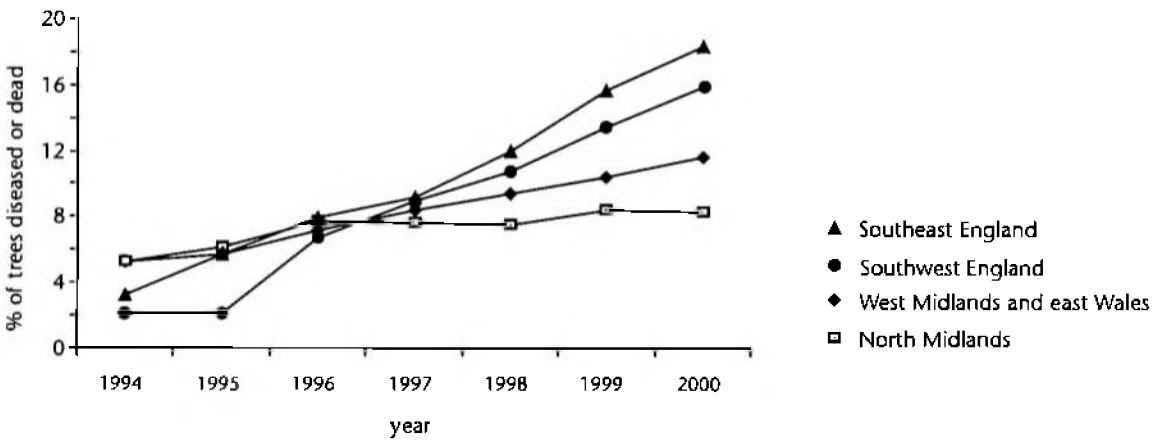
Table 6.1 Data on Phytophthora disease of alder from plots across the southern part of England and east Wales

	1994	1995	1996	1997	1998	1999	2000
Number of trees assessed (n)	1681	1718	1719	1721	1716	1734	1763
Total missing trees since last visit	-	5	47	51	37	29	19
Number observed with Phytophthora	51	63	86	101	112	138	164
Number dead (of which long dead)	22 (7)	28 (14)	40 (23)	44 (24)	54 (30)	59 (33)	61 (40)
Total observed diseased/dead (d)	73	90	126	145	166	197	225
Missing trees since last visit which were previously diseased/dead (m)	-	1	5	7	5	11	8
% of trees observed diseased/dead	43	5.2	7.3	8.4	9.7	11.4	12.8
Annual incidence of disease in year 1 $\frac{(d_i + m_i - d_{i-1})}{(n_i + m_i - d_{i-1})} \times 100$	-	1.09	2.51	1.62	1.65	2.66	2.29

2000. Division of the survey area into four regions shows marked differences in the rate of disease development. It is high in southwest and southeast England and low in the north midlands (Figure 6.2).

Some trees have been diseased for much or all the survey period but have not died. Often this is because they are multi-stemmed and the disease can take a long time to progress to the point at which all the stems are dead. Occasionally there has been definite recovery from the disease. This can take a number of forms. Sometimes one stem on a coppice stool showed symptoms and then died but other stems remained healthy. At other times, individual stems showed complete recovery – severe foliage symptoms subsided and the lesion at the base of the stem callused over.

Figure 6.2 Disease development in 100 km grid squares in four regions of the UK survey area.



The 1994 survey data provided strong support for the role of river water in disseminating the pathogen: disease incidence was 5.4% in trees within 1 m of the river bank as compared to 0.7% in trees growing further away. Moreover all the diseased trees in the latter category were subject to flooding. The 1994 survey also revealed a significant positive correlation between the amount of disease and the mean value for Total Oxidised Nitrogen (TON) in the river water during the years 1992 to 1994 ($r = 0.59$; Gibbs *et al.*, 1999). However, an analysis of the AID over the years 1994 to 2000 in plots with a TON level of more or less than 5 mg l^{-1} has not provided supporting evidence for this effect (J.N. Gibbs, unpublished). Thus the average AID in the 'high nitrate' plots has been slightly lower than that in the 'low nitrate' plots – 2.46 as compared to 2.66.

Austria

Between 1996 and 1998 long-term monitoring plots were established in Austria (T. Cech, Records of the CA). This was not on the basis of a grid as in the UK, but in relation to the known occurrence of dieback or decline phenomena in *A. glutinosa* or *A. incana*. Each plot consisted of 50–60 trees and these were monitored once a year, following a scheme similar to that used in the UK.

In six of the plots Phytophthora disease has been confirmed and the diseased trees showed the classic symptoms: sparse foliage of the whole crown (though not always yellowish) and tarry spots on the stem. On the other two plots, comprising *A. incana* in the riparian forest of the River Danube in eastern Austria (Binderau and Stockerau), there is a more generalised dieback phenomenon and, following investigation, it was concluded that *Phytophthora* was not involved.

In the plots with Phytophthora disease, the incidence of trees with classic symptoms varied, being lowest in a plot containing trees of 80 or more years of age and higher in the other plots where tree age ranged from 15 to 40 years. In the three plots in Upper Austria that have been monitored since 1997 there has been a marked fluctuation from year to year in the percentage of trees showing classic symptoms and very few trees have died (see Table 6.2). It is quite common for trees which show a combination of tarry spots and sparse foliage in one year to show more or less normal foliage with no signs of active stem lesions the following year. Riparian stands showing progressive killing by the disease are the exception rather than the rule in this part of Austria.

France

Quantitative information on the disease has been collected jointly by staff of the Laboratoire National de la Protection des

Table 6.2 Change in the condition of *A. glutinosa* at three sites in Upper Austria: plots consist of 50–60 trees

Site number	Trees with Phytophthora symptoms (%)				Dead trees (%)			
	1997	1998	1999	2000	1997	1998	1999	2000
1	70	44	76	34	8	16	18	18
2	54	52	72	52	0	2	2	2
3	56	20	34	14	0	2	2	2

Végétaux and the Département de la Santé des Forêts (DSF). This has recently been summarised by Streito *et al.* (2002). A substantial study of the impact of the disease in the north and the southwest of the country was conducted in 1998 by the DSF: more than 10% of the trees were found to be either dead or declining. Up to 40% disease was recorded in parts of the northeast. A survey of over 2000 alders at four sites in this area showed that 12% of the trees had 'classic' symptoms of disease, 6% had tarry spots only, 2% had sparse yellow foliage only and 3% were dead.

At Charente in western France, 26 plots, each consisting of 60 trees were established. An alder Phytophthora had been isolated from several of them. Serious dieback was present in 40% of stems and 1% of the stems were dead. The nearer the trees were to the water level, the greater was the degree of damage.

In northeastern France, 14 disease monitoring plots were established in 1998 and 1999 (J-C. Streito, Records of the CA). These were selected to include sites ranging from those on which the disease was having a serious impact to those where it was present at a low level. The number of stems varied from 25 to 91; 817 stems being assessed in total. Data for the six plots which have been assessed each year since 1998 are shown in Table 6.3. A marked deterioration occurred in some of the plots while others showed little change. The latter included the plot at Liverdun where alder Phytophthora was isolated as early as 1996 but where disease levels have remained very low. Across all the plots, an improvement in the condition of individual trees was recorded only rarely.

The disease in non-riparian sites

The disease in woodland and the link with nursery plants

The disease was first found in a small planted woodland in east Wales in September 1993 (Gibbs, 1995). The trees involved were *c.* four-year-old *A. glutinosa* growing on a steep west-facing bank at least 10 m above an adjacent stream and with no possible chance of flooding. Some of the trees showed unmistakable symptoms of Phytophthora disease and the basal lesions were particularly

Table 6.3 Information on alder plots in France established for disease progress assessments

Site	Number of stems studied	Trees with Phytophthora sysmptoms (%)			Dead trees (%)		
		1998	1999	2000	1998	1999	2000
Liverdun[a]	63	3	5	5	0	0	0
Goncourt	65	7	12	7	0	0	0
Niedermodern[a]	57	38	31	27	0	12	7[a]
Mittersheim[a]	63	30	32	35	6	13	12[a]
Harskirchen[a]	63	48	44	53	2	10	12
Gonaincourt[a]	64	50	47	40	11	16	18

[a]The fall in the percentage of dead trees is due to the disappearance of several old dead trees.

striking (Figure 6.3). In August 1996 the disease was found in seven-year-old plants in a woodland at Aberdare Country Park, also in eastern Wales, and examination of the records revealed that both plantations had been established in the same growing season with saplings supplied from the same nursery. Precise details on their origin is not known but it has been discovered that they were imported to the UK probably from Belgium, in December 1988, and then planted out the same winter (J.N. Gibbs, unpublished data).

In 1995 the disease was reported from container-grown alder in a nursery in western England. The nursery conducted its irrigation using water from the adjacent River Teme, on the banks of which were many diseased alders.

Figure 6.3 Basal lesion on 4-year-old *A. glutinosa* growing on a woodland site in east Wales, UK (J.N. Gibbs).

The water was subject to a process of filtration to remove solid material and was then supplied, by means of a trickle irrigation system, to the containers of alders and other broadleaved trees, which also included many alders. Since all the alders were grown from seed in the nursery, the likelihood of extraneous contamination was extremely small. Interestingly the disease was only found in one row of six-year-old *A. incana*; this was despite the fact that there were many other rows of younger container-grown alder in the same part of the nursery.

Important information on the role of nursery plants and disease dissemination has recently come from studies in Bavaria by Jung *et al.* (2000, 2001). In 1998, during an assessment of the disease status in the state, attention was drawn to the presence of severe disease on the River Vils. On investigation it was established that the disease occurred downstream but not upstream of several alder plantings which had been established between 1994 and 1996. A similar situation was found on the Rivers Abens and Ilm, where plantings had been established in 1988 and 1994, respectively, and on the brooks Eschlbach and Gaensemuehlbach. Here the alders had been planted between 1994 and 1996.

Also, in Bavaria, the disease was found in ten new plantations (established between 1988 and 1998) where there was no opportunity of flooding from an adjacent river and where there was no close link with any other alder stand. The influence of topography on disease development was also apparent. The incidence of disease was twice as high in the part of the plantation that lay at the bottom of the site, where the soil was very wet, as in the part higher up the slope, where the soil was merely moist (Jung *et al.*, in press).

To examine the Bavarian situation further, alders were bought in from four private nurseries. The alder Phytophthora was obtained from three of them. From one nursery it was isolated from the soil and roots of all 12

plants. In addition, *P. cambivora, P. citricola, P. gonapodyides* and *P. megasperma* cf. were isolated quite frequently from the plants. By contrast no Alder Phytophthora was isolated from alder plants from four nurseries belonging to the Forestry Authority. A preliminary small-scale survey of private Bavarian nurseries has revealed that none grow their own alders from seed. Instead, one-year-old plants are bought from large nurseries elsewhere in Germany or in other EU countries, and then grown on for a second year before resale. It seems all too possible that this practice could account for the rapid dissemination of the pathogen across Bavaria and, indeed, across Europe. See Brasier (Chapter 5) for further discussion of this point in relation to the different forms of the alder Phytophthora involved.

Other evidence linking the disease with a nursery comes from Sweden and Italy. In Sweden in 1997, the pathogen was isolated from necrotic roots on a sample of one-year-old alders removed from a nursery. This nursery was irrigated with water from an adjacent river but, as yet, the status of the disease on that river is not clear (C. Olsson, Records of the CA). In 2000 the disease was identified in a nursery in Northern Tuscany on one- and two-year-old seedlings of *A. cordata* (Santini *et al.*, 2001). The plants had been grown from seed in that nursery and, at present, it is not known how the fungus might have arrived there.

The disease in shelterbelts

The disease has been recorded in a number of alder shelterbelts in the UK. The first of these was in an apple orchard in Kent, southeast England where trees of *A. glutinosa* and *A. incana*, planted in *c.* 1980, were growing on a light loam several hundred metres from the nearest stream. In 1992 dying trees were observed by the nursery manager and in 1994 the presence of Phytophthora disease was confirmed (Figure 6.4a and b).

Figure 6.4 (a) Shelterbelt of *A. incana* in a Kent apple orchard showing a gap created through the felling of trees killed by Phytophthora disease; (b) base of a tree with tarry spots (J.N. Gibbs).

No explanation for the arrival of the disease on site can be offered. There was no evidence of recent planting that could have lead to the introduction of the pathogen.

An assessment of disease in the two alder species was conducted in June 1994 and the results are shown in Table 6.4. Some caution must be exercised in interpretation, but the fact that the rows of the two species were growing in rows interspersed with each other, indicates that they were probably equally exposed to infection. Therefore the much higher incidence of disease in *A. incana* may be a true reflection of greater susceptibility to disease. The site was reassessed in July 1999 and it was discovered that there had been very little change in the health of the trees during the intervening five years. Although a small amount of active disease could still be seen, few trees had died and some of the trees with old basal lesions had quite healthy and vigorous crowns.

The second site was also an apple orchard, this time in Norfolk, east England. *A. glutinosa* was planted as a shelterbelt in 1970 followed by *A. incana* in 1975 and *A. cordata* in 1980. When assessed in June 1995, the incidence of disease was 1% in *A. glutinosa*, 15% in *A. incana* and 19% in *A. cordata*. Again there was no obvious explanation for the appearance of the disease after many years of successful growth.

The third site was a strawberry farm in Hampshire, southeast England. *A. cordata* had been planted as a shelterbelt in 1987. The principal interest here was that, in 1997, it was found that there was one distinct focus of disease, at the lowest and wettest part of the site. Investigations showed that the first trees had died in 1993, when they were six years old, and that there seemed to have been a fairly steady progress of disease from tree to tree, at an average rate of 2.5 m per year. Some of the trees were excavated but it could not be determined with confidence whether the pathogen had moved from tree to tree via root contacts or by means of zoospores (D. Lonsdale, personal communication). There had been some recent planting of alder but not near the place where the disease occurred .

Conclusions

Early field investigations on the disease pointed clearly to the vulnerability of alders growing on riparian sites and these observations have been supported by more recent studies. The pathogen can evidently operate under very varied aquatic conditions and there is a requirement for detailed investigation into the character and behaviour of its waterborne propagules. *Inter alia*, this needs to include research on the importance, for infection and

Table 6.4 Extent of disease in orchard shelter belts of *A. glutinosa* and *A. incana* in Kent, UK

Species	Number of trees				Cumulative percentage affected by Phytophthora disease
	Assessed	With Phytophthora crown symptoms	Dead due to Phytophthora	Felled due to Phytophthora	
A. glutinosa	611	50	1	11	10
A. incana	1569	285	14	158	29

host invasion, of variations in water level – whether these are transient, as when a river is in spate, or permanent, as when a river is dammed.

One point to emerge from the survey work is that the rate of disease development can vary from place to place and from time to time. In some localities, the disease is intensifying inexorably, and there is every indication that this process will continue until very few alders remain. In others, the level of disease has remained relatively static and some trees have recovered from their symptoms. There are indications from a number of sources (see also Streito, Chapter 4) that the period 1993 to 1995 was one in which the disease increased rapidly and that these years were followed by a period during which the disease became somewhat more quiescent. Further investigation is needed into the roles of variation in host resistance and of variation in fungal pathogenicity (see Brasier, Chapter 5) on the pattern of disease development on particular sites. The influence of environmental factors such as nitrate concentration in river water requires experimental investigation. Preliminary consideration of disease data from a few rivers indicates that it might be valuable to study the effects of sudden changes in nitrate levels.

Work on non-riparian woodland sites quickly drew attention to the role of planting stock in pathogen dissemination. There is now no doubt that it has reached new areas on young alder plants. This finding has clear implications for any attempts to limit further spread of the pathogen (see Van Dijk and Gibbs, Chapter 8). More needs to be known about the status of the alder Phytophthoras in tree nurseries and, in particular, on the likelihood of their being transported on plants other than alder. This could be the means whereby the disease reached the shelterbelts described above.

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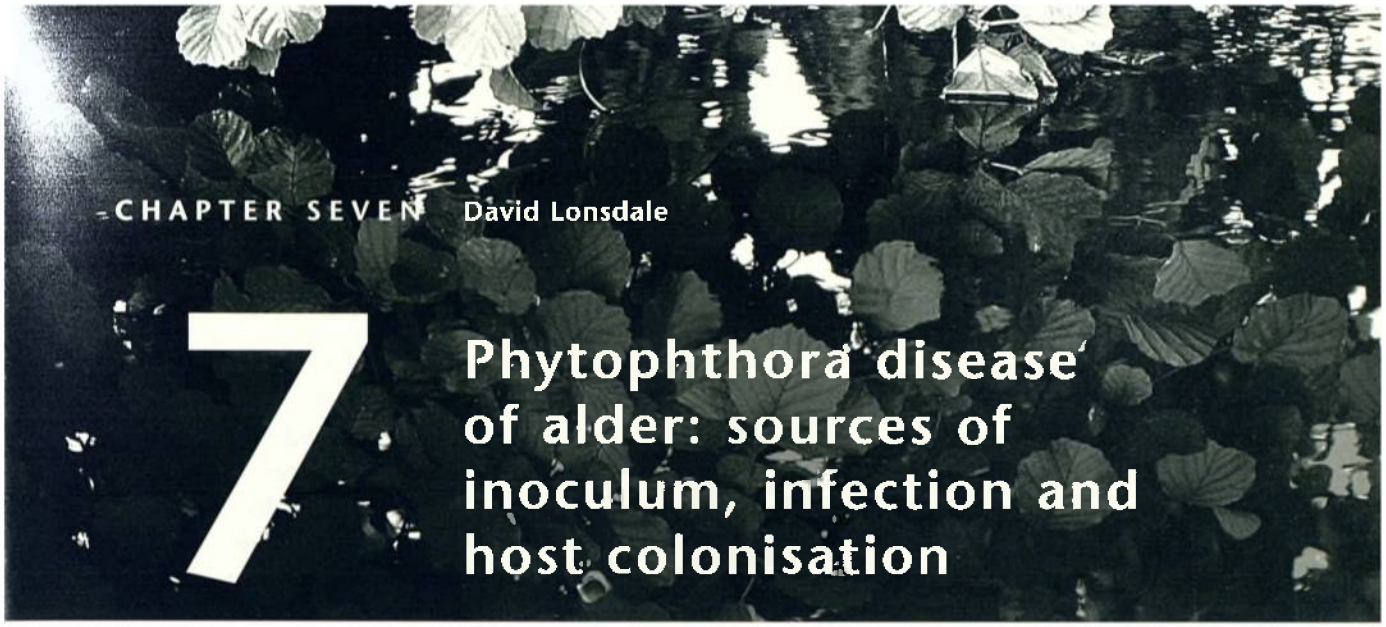


CHAPTER SEVEN

David Lonsdale

7 Phytophthora disease of alder: sources of inoculum, infection and host colonisation

Introduction

In analysing a disease cycle, we need to understand the processes whereby host tissues are infected and subsequently become a source of inocula for further dissemination of the pathogen. In the case of the Phytophthora disease of alder, these processes are imperfectly understood but some insight into the disease cycle can be gained by considering first the potential inoculum sources and then the available information on infection and host colonisation. In this context it should be borne in mind that any generalisations that we may make about the disease cycle might not apply to all forms of the alder Phytophthora, which comprise a number of different and in some cases unstable taxa with potentially different behaviour patterns (Brasier, Chapter 5).

Inoculum sources

Bark tissue

One obvious source of inoculum is the necrotic bark tissue (cortex and phloem) on the stem bases and woody roots of trees with typical disease symptoms. The main evidence for the potential of such bark as an inoculum source is the ability to isolate alder Phytophthoras from it using baits and agar media. The capacity of bark inocula to cause infection of test plants has not been directly studied, but T. Jung (Records of the CA) found that a shredded bark lesion produced potentially infective propagules in the form of abundant sporangia after a few hours in water. On the other hand, workers in this field have commonly reported a lack of success in attempting to isolate the fungus from bark tissue (Streito, Chapter 4). One factor has undoubtedly been a failure to provide suitable conditions for isolation but another could well be

absence of the fungus from certain zones within the necrotic tissue. Even among tissues that have been invaded by the pathogen, there will be considerable variation: some will have been colonised very recently, others will have been colonised months or even years beforehand. Knowledge that the fungus can be isolated most readily from recently killed tissue suggests that such tissue would be the most potent source of infection. The frequent failure to isolate the fungus from long-dead tissue may also be partly due to the presence of competing micro-organisms and not necessarily an indication that the fungus is no longer viable.

C. Olsson (Records of the CA) has been able to isolate the fungus easily by direct plating of material from inactive blackened lesions, provided that excess moisture is removed so as to discourage bacterial growth. T. Jung (Records of the CA) has also shown that the fungus can be isolated from long-dead tissues. Similarly, J-C. Streito (Records of the CA) has conducted some studies in which samples, comprising both bark and underlying wood, were removed from dead trees and placed in soil on the riverbank. At monthly intervals, sub-samples were retrieved and split open. Successful isolation from the exposed surfaces showed that the fungus could be retrieved for at least three months.

Root tissues

Compared with bark, fine roots have not been much investigated as a potential source of inoculum. D. Lonsdale (Records of the CA) found that fine roots previously infected in a zoospore inoculation experiment produced small numbers of sporangia of the alder Phytophthora after immersion in water. Although this observational evidence has not been supplemented by any quantitative studies, it supports the idea that infected fine roots could be an important form of inoculum. In many Phytophthoras, the formation of sporangia from such roots can allow rapid cycles of repeated infection and fresh sporulation within the fine root system under suitable conditions such as flooding.

Particles and propagules in soil and water

In assessing the roles of different types of inocula, it is necessary to consider not only necrotic tissues in a recognisable form, but also soil and water which may contain comminuted host material or free-existing propagules. The research of C. van Dijk (Records of the CA) in the Netherlands is particularly interesting in this context, as it indicates that soils in natural stands of *Alnus glutinosa* may contain infective material. He developed a technique for detecting those soil-borne Phytophthoras that might have the potential to influence the composition and dynamics of forest stands. This involved inoculating test seedlings in hydroponic culture by introducing suspensions of the test soils into the culture solution. Having previously detected *P. citricola* in Meertje de Waal, a coastal peat bog area, van Dijk tested soil samples from alder carr in 1995 from Die Wieden Nature Reserve, selecting ten locations where there had been no human disturbance for at least 25 years. There was no evidence of Phytophthora disease of alder in De Wieden, although some trees were showing various types of dieback. However, when tested with the hydroponic baiting method, soil from four of the ten locations sampled yielded a variant of the alder Phytophthora, now known as the Dutch variant (see Brasier, Chapter 5). The exact nature of the infective material in these soils remains uncertain, as the preparation of the samples would probably have allowed the survival of all stages of the fungus. Indeed, the samples may have included infected root material.

Dormant survival

The form in which the fungus survives in long-dead tissues, and perhaps also in soil, has not been investigated but knowledge of other

Phytophthoras might suggest the involvement of oospores, which the alder Phytophthora produces abundantly in culture. However, this view was dealt a significant blow by the work of Delcán and Brasier (2001). While studying sources of variation among alder Phytophthoras they attempted to induce oospore germination on distilled water agar but without success, even after eight weeks in some cases. Under the same conditions, isolates of other *Phytophthora* spp. showed between 0.5 and 54% germination. In the same study, a tetrazolium bromide test indicated that a high proportion of oospores (up to 69% in 'standard' isolates of the alder *Phytophthora*) were non-viable.

In view of the findings of Delcán and Brasier (2001), it seems likely that oospores have no role either in dormant survival or in dissemination of the fungus. Even their very existence in nature has not been established. Although D. Lonsdale and J. Engels (unpublished) observed them in small numbers within artificially inoculated wood blocks under laboratory conditions, they found none in a preliminary examination of the wood or bark of alders affected naturally by the disease in Worcestershire, England.

The dormant survival of some Phytophthoras is mediated by chlamydospores or by thick-walled hyphal cells. Although the alder Phytophthora has not been observed to form chlamydospores, it can produce thick lateral hyphae which could perhaps function as dormant propagules, as in the case of *P. cactorum* (Blackwell, 1943). The latter species, like several other Phytophthoras, can also form sporangia with some capacity for dormant survival, but there is no evidence of sporangial dormancy in the alder Phytophthora.

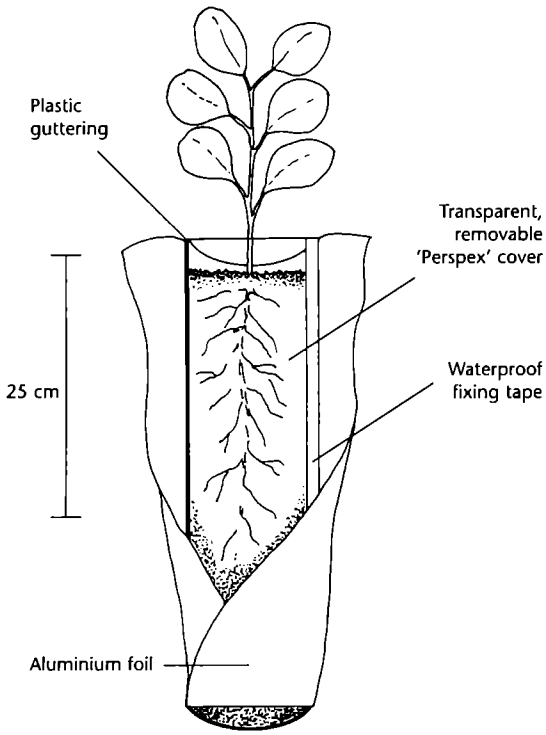
Although the pathogen has some capacity to survive for at least three months in necrotic tissue, very little is known about the effect of ageing on the potency of such material as an inoculum source. Similarly, it is not known whether fungal survival within seasonally inactive lesions is prolonged enough to allow renewed lesion extension when conditions become more favourable for the invasion of host tissues. There is, however, observational evidence that perennial cankering may develop due to recrudescence of infections, as shown by successive patterns of callus formation in stem bases (D. Lonsdale, unpublished).

The persistence of the pathogen in necrotic tissue might indicate that it could also survive in plant fragments within soil, but this has not been directly investigated. Delcán and Brasier (2001) did, however, assess saprotrophic survival by mixing soil with macerations of apples colonised by the alder Phytophthora and *P. gonapodyides* in various proportions and the pathogen could not be re-isolated after one month.

Zoospores and other agents of infection

Infection processes in the Phytophthora disease of alder have not been observed in detail and there remains a need to determine which forms of the fungus are able to penetrate the host surface. The question as to which parts of the host are susceptible to infection will be discussed in the next section. In Phytophthoras generally, zoospores are a major agent of infection and it seems probable that they play a part in this disease. Cultures of the fungus can readily be induced to produce abundant sporangia and zoospores when flooded, at least in the presence of commonly occurring types of bacteria (D. Lonsdale, unpublished). The zoospores, like those of other species, show a strong chemotactic or electrotactic attraction to the cell elongation zones of roots of various plant genera, especially alder (D. Lonsdale, Records of the CA). When applied to the unwounded root tips of young alders in a mini-rhizotron system, zoospores caused infection and subsequent necrosis of the fine roots (see Figure 7.1).

Figure 7.1 'Mini-rhizotron', constructed from a section of plastic guttering. The transparent Perspex cover allows viewing of the root system and can be hinged open after removal of the fixing tape from one side. Aluminium foil excludes light from the roots, which are encouraged to grow towards the cover by keeping the unit inclined at 10° to the vertical.



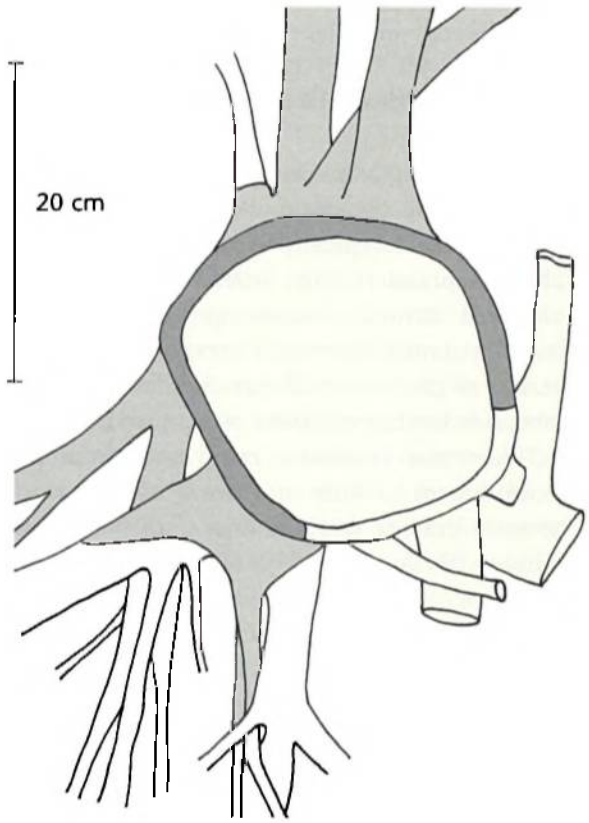
The involvement of zoospores seems likely in an accidental cross-inoculation of test plants reported by S. Werres (Records of the CA), in which glasshouse irrigation water had become contaminated with the fungus. Zoospores would seem to be the most likely form of inoculum to be transported in this way and then to cause infection. In the same study, the test plants had been successfully inoculated using a culture macerate, but in such cases it is not possible to determine whether zoospores are involved, as various forms of the fungus are likely to be present.

Infection courts

As already mentioned, fine non-woody roots have been infected without wounding in an inoculation experiment using zoospores, but the importance of this mode of infection has not been studied under natural conditions. Although it remains eminently possible that fine root infection is both epidemiologically and pathologically important, such infection is for obvious reasons not conspicuous in the field. Instead, infection of the stem base and major roots is by far the most frequently observed type of lesion development. Some confirmation that this zone of the tree is indeed the main seat of overt infection was obtained by mapping the extent of necrotic bark above and below ground on eleven naturally infected trees in Oxfordshire, Hampshire and Surrey in southern England (Figure 7.2; D. Lonsdale, Records of the CA). In all the diseased trees examined in this study, bark necrosis was more extensive on the upper sides of the main roots than on their lower sides. This pattern may suggest that the upper sides of these roots and perhaps the stem base are the main infection courts. There is, however, another plausible explanation, i.e. that these areas are the most susceptible to tissue invasion. If this is true, primary infection could start in a distal part of the root system and then rapidly develop once the fungus had reached the base of the tree. Observations by T. Jung (personal communication) in Bavaria are consistent with such a view. He noted that, in a plantation on a site not subject to flooding, the pathogen took eight years from planting in 1991 to reach the stem base but then, in several trees, grew more than 40 cm up the stem during 2000. Invasion from roots to the stem base seems to have occurred in the study by S. Werres (Records of the CA), who found basal lesions in test plants which had been root-dipped in an inoculum before planting in waterlogged soil.

Of the trees examined by Lonsdale (*op. cit.*), most had at least one root showing distal

Figure 7.2 The cut stem base of one of the trees assessed for the initiation and spread of root and bark necrosis (River Wey, Surrey, UK, 1997). This tree showed tarry spots but only slight foliar symptoms. The shaded areas show the extent of bark necrosis at the cut surface and necrosis in the attached roots.



necrosis and therefore did not provide clear evidence that infection had started only in the basal zone. Elucidation of the direction of fungal growth along the distally affected roots might have helped to provide evidence but this was not possible, as such roots had invariably become entirely necrotic due to general dysfunction which obscured the margins of the lesions. In one tree, however, distal infections of woody roots were entirely absent and can thus be deemed to have played no part in the invasion of the basal zone in this instance.

Although the basal zone may well be the main seat and perhaps the origin of infection, the infection courts have yet to be accurately identified. As mentioned above, the frequent concentration of necrosis on the upper sides of the main roots, rather than on their lower sides, does not necessarily indicate exactly where infection is being initiated. The stem base and the upper sides of its attached roots are, however, the zones most likely to come into contact with inoculum in the form of water-borne spores or infective debris, especially during flooding.

Although it seems likely that trees can acquire primary infection via infective material in soil and water, the possibility of transfer of the fungus via root contacts between trees also needs consideration. One site where this mode of spread may have occurred was a single line of *A. glutinosa* planted as a shelterbelt on sloping land around a strawberry field in southern England (see Gibbs *et al.*, Chapter 6). J.N. Gibbs and D. Lonsdale (unpublished) were able to identify a water drainage channel as the likely route for the primary spread of inoculum, but observed that symptoms of disease were appearing sequentially in trees not only down the slope but also uphill, i.e. against the flow of water.

As far as the exact points of host penetration are concerned, no investigations have yet been completed. The chemotactic responses of alder Phytophthora zoospores, together with their infective capacity, indicate that root tip entry probably occurs as with other root-infecting Phytophthoras. Entry via wounds on roots seems slightly more doubtful as a mode of infection by zoospores, in view of the observation by D. Lonsdale (Records of the CA) that there was only very slight chemotaxis towards the cut surfaces of alder seedling roots placed in a zoospore suspension. Root wounds were present in the plants successfully inoculated by S. Werres (Records of the CA), whose findings are mentioned above in relation to progression of the fungus from roots to the stem base.

The frequent occurrence of the fungus in the bark of the stem base and major roots suggests that it may be able to penetrate the bark directly, although this mode of entry has not been shown to occur in this disease. Bark in this region often produces adventitious roots in moist conditions, and such roots might serve as an easy means of entry for germinating zoospores. Observation of young lesions by T. Jung (personal communication) support this view. It is also interesting to speculate that lenticels, whose cells tend to proliferate under wet conditions, may provide another infection court. However, when cut alder twigs were used as bait by floating them in watercourses at Liverdun and Harskirchen in France, infection occurred only at the cut ends, and then only very occasionally (see Streito, 2002 and Chapter 4, and Brasier, Chapter 5).

It remains unclear whether the alder Phytophthora can establish infection via wounds in bark under natural conditions, notwithstanding the successful use of such wounds for artificial inoculation (Gibbs, 1995). Bark wounds are an important infection court in some Phytophthora diseases, such as stem canker of avocado caused by *P. citricola* (El-Hamalawi and Menge, 1994). It has, however, been reported that *P. cinnamomi* is able to penetrate the suberised bark of *Eucalyptus marginata* in Western Australia during flooding of stem bases (O'Gara *et al.*, 1996).

Host invasion

Once the fungus has entered the host, its ability to develop within the tissue is of great importance in determining the type and severity of disease expression.

Invasion via fine roots

As discussed above, primary infection can occur in fine roots, but it is not known whether the fungus can then spread by tissue invasion to larger roots. In the mini-rhizotron study reported by D. Lonsdale (Records of the CA), necrosis developed in fine roots following a zoospore inoculation, but did not usually progress beyond the junction with a parent root. Field observations of trees in very wet conditions suggest, however, that the fungus may be able to grow from infected adventitious roots directly into the stem (page 69).

Invasion of inner bark

Once the fungus has entered the bark of the stem base or the main woody roots, where overt disease is typically concentrated, it seems able to spread rapidly. Several workers have observed naturally occurring lesions, which had apparently developed enough in a single season to girdle a small tree. Similarly, experimental wound inoculations of stem bark (Figure 7.3) have also resulted in rapid invasion in the axial direction, with an average extension of about 1 cm per week at one of the test sites (Gibbs, 1995).

Figure 7.3 Lenticular stem lesion, induced by wound-inoculation of a young *A. glutinosa* with the alder Phytophthora (J.N.Gibbs).

Upward development of basal lesions usually ceases at between two and three metres. It seems likely that stress-dependent pathogens, such as *Valsa oxystoma* (see Cech and Hendry, Chapter 3), developing within dysfunctional tissue above the lesion, may act to prevent further upward spread.

Factors affecting rate of the invasion of host tissues

Several workers have reported that the active development of stem lesions in naturally infected trees often seems to be seasonal, with spreading lesion edges being found more in the spring and autumn than in summer or winter. The apparent arrest or retardation of lesion development in summer may be partly due to the relatively poor heat-tolerance of the alder *Phytophthora*. The 'standard' form has an optimum for growth on artificial media below 25°C and a maximum below 30°C (Brasier *et al.*, 1995). A temperature effect would, however, not explain summer inactivity in the cooler regions of Europe, nor in cooler-than-average summers elsewhere. Indeed, there have been cases where spreading lesions have been found during summer.

The effect of summer temperature has not been investigated *per se*, but Gibbs (1995) found that the results of wound-inoculating young trees seemed to differ according to summer temperature in southern Britain. By wound-inoculating six-year-old *A. glutinosa* saplings at a valley-slope site in Gwent, South Wales in 1994, he obtained lesions which developed rapidly between June and October. In the course of a provenance trial of two-year-old *A. glutinosa* in southern England, he was again able to induce lesion development as judged by discoloration of the bark near the wound-inoculation sites at the stem bases (see Gibbs, Chapter 8). When, however, hot weather occurred in August, development of the lesions was arrested.

At the other extreme, low temperature will obviously slow down the potential growth rate of the fungus, but it is not known whether lesion development can be arrested as a result. As arrested lesions are very common, it seems possible that factors other than the influence of high or low temperature on the pathogen may also be important. In this context host resistance is of interest, in relation both to inherent seasonal variations and to possible impairment of resistance by physiological stress. Evidence of inherent seasonal variation was shown by Brasier and Kirk (2001), who inoculated the bark of freshly collected alder logs with various isolates of the alder Phytophthora and incubated them at a standard temperature of 20°C. The pathogens developed much more rapidly in logs cut between July and October than in those cut between November and March. Development did not occur at all in logs cut in April (see Brasier, Chapter 5).

Evidence of the impairment of host resistance by stress was shown by T. Cech (Records of the CA), who inoculated plants after maintaining them for 30 days at soil moisture contents of 15, 20 and 30%. In the post-inoculation period, during which normal watering was resumed, no differences in lesion development occurred between these groups of plants. However, when plants in a later inoculation experiment were pre-stressed at a soil moisture content of 7 to 10%, they produced lesions over twice the length of those kept at 30%.

Conclusions

Although many details of infection biology remain to be investigated, the available evidence, both from field observations and from experimental work, provides a basis for the following tentative concept of the disease cycle and the conditions that favour its progress. In water or in wet soil, sporangia and hence zoospores of the pathogen develop from

mycelia within infested plant tissues. Under the same wet conditions, chemotaxis of the zoospores leads to their accumulation on susceptible host surfaces, including root tips and perhaps also lenticels and wounds on bark. When the fungus colonises fresh tissue via sites of zoospore infection, fresh crops of sporangia and zoospores may be produced.

Infection of fine roots in the more distal parts of the root system may lead to a general loss of absorptive function and some consequent decline in the overall health of trees without necessarily leading to overt disease. On the other hand, infections that develop in the bark tissue of the major woody roots and the stem base can lead to girdling and rapid death. If such lesions fail to girdle the tree within a single season, host resistance or seasonal inactivity of the fungus may allow some remission or even elimination of disease development, characterised by the formation of occluding bark and wood around the lesions.

Another pattern that seems to emerge from observations by several workers is the involvement of flooding at sites where a high proportion of trees have become overtly affected. Flooding is known to favour pathogenesis by *Phytophthora* spp. in various ways. It induces microaerophilic conditions, which cause physiological stress in host tissues and thus increases their susceptibility to attack. Moreover, root exudates produced during anaerobic respiration tend to be highly attractive to *Phytophthora* zoospores, repeated generations of which can develop very rapidly from submerged infested tissue. Also, floodwater contains stagnant or slowly flowing zones, in which chemotaxis and accumulation of zoospores is not hindered by fast currents, as might be the case within the main channel of the river.

If flooding is an important factor, it may explain why the incidence of disease at some British sites is very high and yet has increased quite slowly among riparian alders generally (Gibbs *et al.*, Chapter 8). Thus, although the riparian habitat might seem ideal for the development of disease, there is reason to hope that it will not always provide conditions for the occurrence of severe mortality.

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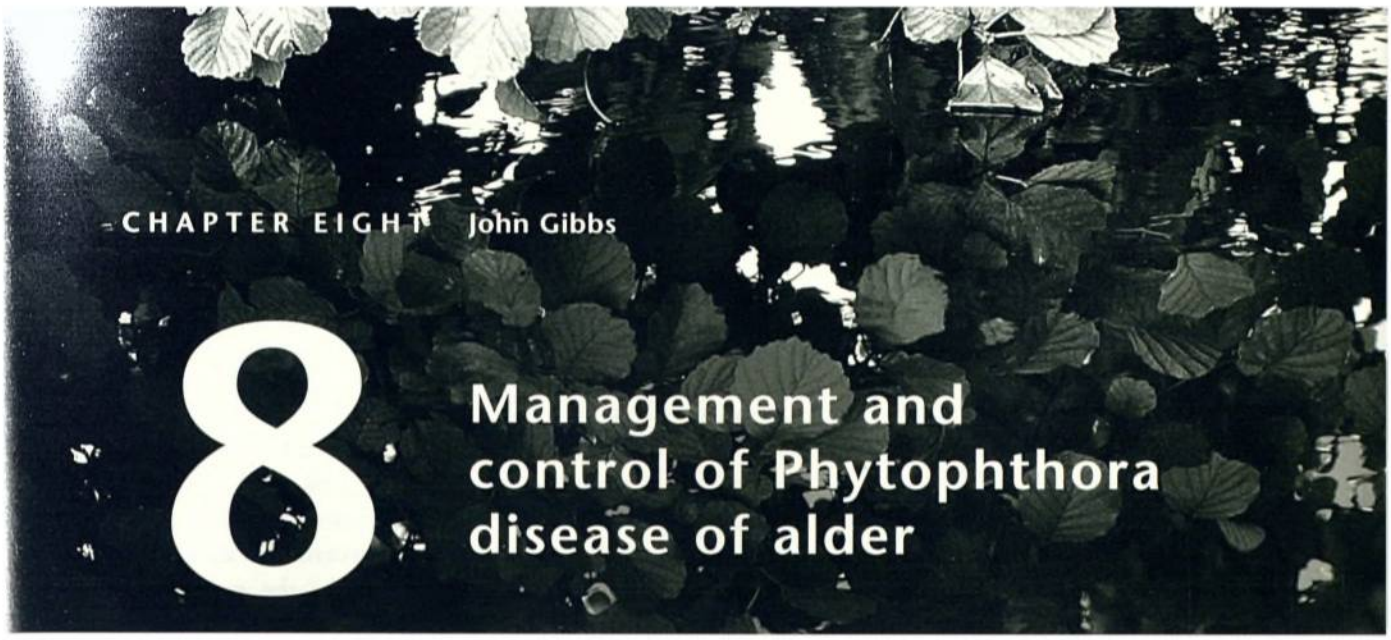

CHAPTER EIGHT John Gibbs

8 Management and control of Phytophthora disease of alder

Introduction

The symptomatology, distribution and impact of Phytophthora disease of alder have been described by Streito (Chapter 4) and Gibbs *et al.* (Chapter 6). This chapter is concerned with disease management and control. The terms 'disease management' and 'disease control' are sometimes used interchangeably. However, the former term does give a wider perspective than the latter and allows consideration of approaches that might otherwise be neglected. For example, if evidence indicates that alders growing on low-lying ground beside a river are more vulnerable to the Phytophthora disease than trees on the bank itself (see Gibbs *et al.*, this volume) then this might offer a useful approach to disease management on certain sites.

With any destructive new disease, issues of management and control are vitally important. Approaches to the problem can be short term or long term. When the Phytophthora disease of alder was first discovered in 1993 (Gibbs *et al.*, 1994), it was thought that it might be very restricted in distribution and that measures to prevent its wider dissemination might be of paramount importance. These issues still remain highly significant (see van Dijk and Gibbs, Chapter 9) but there is inevitably just as much interest in the development of measures to ameliorate the situation in localities where the disease is well established. These measures can involve methods of seeking to maintain existing populations of the host, whether partly diseased or still healthy, and methods of seeking to minimise the occurrence of disease in any new host populations that develop naturally or that might be established by planting.

Cultural measures

Almost from the outset it was realised that a 'sanitation approach' based on destroying diseased trees was not feasible. Such approaches are rarely successful in natural or semi-natural host populations and the possibility of success in this case was further reduced by the fact that diseased trees do not show crown symptoms until most of the bark at the base of tree has been killed (see Streito, Chapter 4). By this time the affected trees are likely to have produced inoculum of the pathogen for months if not years (see Lonsdale, Chapter 7). Moreover the process of tree removal, say by winching the roots out of the ground, would not only be extremely difficult but also highly destructive to a riparian habitat.

By contrast, there has been considerable interest in the idea that some amelioration in the situation could be achieved by coppicing the affected trees. Coppicing is a traditional method of managing riparian alder and any new growth arising from affected stools would be expected to gain greatly from the light and space that the removal of dead or severely diseased stems would afford.

A number of studies have been established but most are still at an early stage of development. The first one was initiated along a section of the Hadley Brook in Worcestershire, England in the summer 1996. At that time all the stems (20–30 year old) were cut from a series of coppice stools in different stages of health along a 150 m length of brook. The results after four years are shown in Table 8.1. Some re-growth occurred in all categories of stool. Not surprisingly, the proportion of stools that gave rise to such re-growth was much higher in those that were healthy or still had some healthy stems at the time of coppicing than in those where all the stems were dead or were showing disease symptoms. Regrowth in the former group was also more abundant as judged by the number of shoots over 2 cm diameter at the base. Often it was very vigorous with heights of 6 m being commonly recorded. In the latter group, height growth tended to be less, usually in the range of 2–5 m. Nevertheless, some of the badly affected stools were showing sustained growth.

Clearly the vulnerability of the new stems to new disease is a matter of great importance. Disease symptoms in the regrowth were noted in 1999 and these increased somewhat in 2000.

Table 8.1 Regeneration of *A.glutinosa* growing on the Hadley Brook in Worcestershire, UK following coppicing in summer 1996

Condition of trees at the time of coppicing	Total number	Number with live regrowth in 2000	Mean number of shoots per stool >2 cm diameter at base	Number of stools with signs of new disease in 2000
All stems dead	8	3	2	0
All stems visibly affected or dead	13	8	4	2
Some stems healthy, others affected or dead	7	5	9	1
All stems healthy	21	20	17	9

At this time disease was present in 12 of the stools, although it typically affected only a very small proportion of the shoots. Continued monitoring is clearly required. Adjacent to the study area is a section of brook where no coppicing was carried out. Here there were many stools showing feeble epicormic growth on otherwise dead stems. In addition, several dead stools had fallen into the brook resulting in bank erosion that would not otherwise have occurred.

Two other studies should be mentioned. The first study, in northeast France, was initiated in 1998 (Table 8.2). Five plots were established. At Niederstinzel and Bischtroff, the coppicing had been conducted in 1997 specifically because of the disease, whereas at the three other sites, the trees all appeared to be healthy. The results are similar to those observed in the UK: regrowth was more abundant and vigorous where the trees were healthy at the time of cutting, but some severely diseased stools produced healthy shoots. As yet Phytophthora symptoms have not been observed on the regrowth.

In the second study, on the river Salm in Belgium, various treatments were applied in the autumn of 2000 to a number of stools carrying 20-year-old coppice. In some cases all the stems were cut; in others only those with disease symptoms. Some stools have been left uncut to act as controls. Progress of the regrowth will be monitored in subsequent years.

Chemical treatment

No serious experimental consideration has been given to this approach as yet. The most promising candidate material is phosphite (phosphonate) – normally as the potassium salt (Coffey, 1991). However despite its relatively benign character (it is readily oxidised to phosphate), there must be some caution about using it on the edge of an aquatic ecosystem. Nonetheless, recent experience in Australia with trunk injection and foliar spraying to reduce the damage caused by *Phytophthora cinnamomi* to various native plants in western Australia (Hardy, 2000) suggests that there is a case for further investigation, especially where valuable individual trees concerned.

Host resistance

From the outset there has been considerable interest in the idea that 'useful' resistance to the

Table 8.2 Regrowth stools of *A.glutinosa* after coppicing at five sites in northeast France

Plots	Number of coppice stools	% showing regrowth in 1999	Phytophthora isolated on the site
Pange	14	100	No
Hirtzbach	53	100	No
Goncourt	27	100	No[a]
Niederstinzel	19	58	No[a]
Bischtroff	94	62	Yes

[a]An Alder *Phytophthora* not isolated on the site but isolated upstream and downstream.

disease might occur within the alder population. To date, emphasis has been focused on *A. glutinosa* as being the most important of the European species. In several countries the presence of conspicuously healthy trees in localities where the general incidence of disease is high, has led to the hope that these might be resistant genotypes, although there has also been a recognition that 'disease escape' is likely to play a considerable part in determining the health of any particular tree. To date, the principal formal study has been that conducted in the UK at the Forestry Commission Research Agency, Surrey on a comparison of provenances of *A. glutinosa* collected from across Europe.

In brief, seeds from various sources of *A. glutinosa* were obtained from the Institute for Bosbouw en Wildbeheer at Gerraardsbergen in Belgium in March 1995. These were germinated and grown on in containers, and in July 1996 the 15-month-old seedlings of each provenance, now 50–100 cm in height, were inoculated with alder Phytophthoras (three isolates of the fungus each being used on five plants). Inoculation was conducted by introducing mycelial inoculum into a bark wound at the base of the stem. Bark necrosis was visible within a few days and after three weeks the length of each lesion above the inoculation point was measured (see Table 8.3). Statistical analysis demonstrated that

Table 8.3 *Phytophthora* disease in various provenances of *Alnus glutinosa* in experimental studies in the UK

Provenance	Mean[a] lesion length (cm) on 15-month-old seedlings 3 weeks after inoculation		Percentage of 5½-year-old saplings developing disease following exposure to natural infection at two sites			
			River Clun		River Rother	
Estonia	6.8	a	6%	(16)[b]	-	
Germany	5.3	ab	25%	(16)	33%	(18)
USSR	5.3	ab	31%	(16)	38%	(16)
Lithuania	5.2	ab	13%	(15)	33%	(15)
France	5.1	ab	13%	(15)	56%	(18)
Poland	4.7	bc	14%	(14)	56%	(18)
Belgium	4.1	bcd	12%	(17)	44%	(18)
Hungary	4.0	bcd	0%	(10)	-	
Austria	3.3	cd	0%	(15)	39%	(18)
Corsica	3.2	cd	-		-	
Greece	3.1	cd	7%	(14)	47%	(17)
Yugoslavia	3.1	cd	7%	(14)	47%	(17)
UK (England)	3.0	cd	31%	(16)	59%	(17)
UK (Wales)	-		-		29%	(14)
The Netherlands	2.8	d	12%	(17)	53%	(17)

[a]Based on 15 replicates: means followed by the same letter are not significantly different at $P = 0.01$.
[b]Figures in parentheses indicate number of saplings exposed to natural infection.

there was a significant effect of seed source at $P = 0.01$, with provenances from the Netherlands, the UK, Yugoslavia, Greece, Corsica and Austria showing the smallest lesions.

During the dormant season of 1995/96, saplings from the same batch of seedlings were planted out in randomised plots at sites subject to flooding alongside the river Rother in West Sussex and the river Clun in Shropshire. Diseased trees were present upstream of the experimental sites on both rivers and, on the Clun site, a planting of five-year-old *A. glutinosa* had been felled early in 1995 because of losses through disease.

Symptoms first appeared at the Clun site in 1998 and at the Rother site in 1999. It was at the latter site that it made its greatest impact, with nearly 45% of trees showing symptoms by August 2000. At the Clun site the equivalent figure was 14%. Data for both sites are shown in Table 8.3 and from this it can be seen that, with the exception of the Hungary provenance that was only present at Clun, disease occurred in all provenances at one site or the other. At the Rother site the incidence of disease ranged from 29% to 59%. There was also no indication that the provenances that showed least disease following artificial inoculation were any better than the others in terms of their response to natural infection.

Discouraging as these results are, it should not be concluded that there is no hope of finding useful resistance within *A. glutinosa*. The field experiments will be monitored further with a view to determine if individual trees possess useful resistance even if there are no significant differences between provenances. There also seems to be considerable scope for a search for resistant individuals in those parts of Europe where the natural disease pressure is high. Such work is now being planned in several countries. For example in June 2001, 150 cuttings were taken from each of 30 apparently healthy and seven diseased *A. glutinosa* trees growing on the banks of six Bavarian rivers (Große Vils, Glonn, Gerolsbach, Paar, Schmutter and Zusam), and rooted in a greenhouse under mist. They will be tested for resistance to the alder Phytophthora as soon as possible. All the apparently healthy trees were subject to seasonal flooding and were close to diseased or dead alders. From the presence of healed basal lesions, it seemed probable that some of them had been infected in the past. The disease has been present on the river systems for at least 10 years (T. Jung, unpublished).

Conclusions

Few conclusions can be drawn at this stage. However, given the nature and location of the host population it is unlikely that fungicides will have a major role to play, although phosphite could be of use on specimen trees. The coppicing studies have given reasonably encouraging initial results but it is much too early to determine the extent to which new growth will remain free from disease. Although the studies on resistance conducted so far have been disappointing, this remains an important long-term approach to the disease. New initiatives in this field are to be commended.

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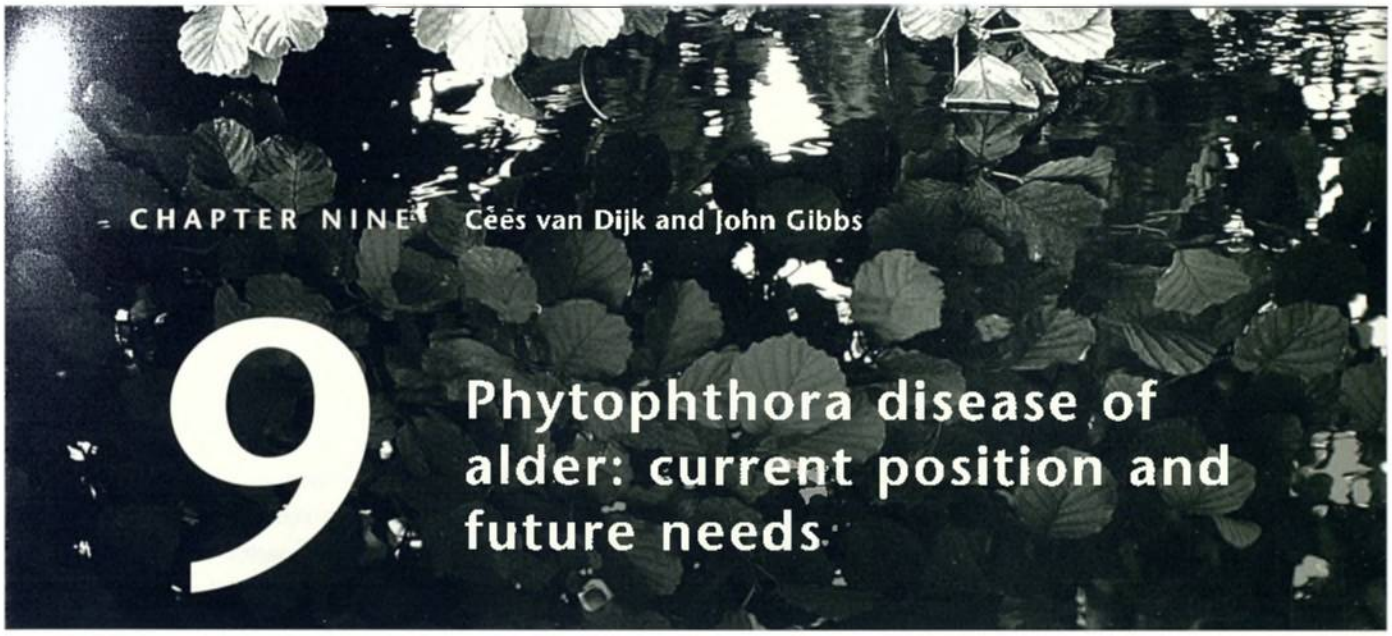

CHAPTER NINE

Cees van Dijk and John Gibbs

9 Phytophthora disease of alder: current position and future needs

Overview

As set out in the preface to this publication, the objectives of the Concerted Action were:

1. To determine if the spread of the disease within Europe can be limited.
2. To make recommendations on disease management and control.
3. To identify research requirements.

In this chapter we attempt to draw the threads of information together to see how far these objectives have been met.

It should be said at the outset that the Concerted Action has played an extremely beneficial role in the sharing of expertise and in the dissemination of new research results. For example, colleagues in different countries have learned both from each other's failures and each other's successes in the isolation of the pathogen. Rapid progress has been made in a way that would otherwise not have been possible. Also, the opportunity to see the disease at sites in different countries during the workshops, has been of great value in interpreting the symptoms and in improving perceptions of disease manifestation. This has been helpful to all the partners – not least to those coming from countries where the disease had not been identified.

The current status of the host and the disease

As outlined in Claessens (Chapter 2), alders are vitally important European trees. This is due to their diversity of characters which not only enables them to establish themselves as pioneer trees on

a wide range of sites but also allows them, under some circumstances, to play a significant part in climax forests. The black alder, in particular, has played a dominant role in the development of forest ecosystems since the last ice age, and despite reductions in abundance with the drainage of wetlands and the clearance of flood-plain woodlands, still occupies large areas. Of special importance are the riparian populations which make a major contribution to the stability of river banks. All four European alder species are important in the establishment of woodland on difficult sites. Crucial here is their ability to establish themselves quickly and to improve site fertility due to nitrogen-fixing abilities of the symbiotic actinomycete *Frankia*. Alder ecosystems have special contributions to make to biodiversity and, in some circumstances, alder is important as a source of timber.

Apart from the Phytophthora disease, European alders are largely free from major pest and disease problems. Quite conspicuous dieback can be caused by certain stem-boring insects. Also drought has often been implicated as a cause of damage (Cech and Hendry, Chapter 3). During the first half of the 20th century, a problem known as 'Erlensterben' aroused concern and was never entirely explained. An inappropriate choice of provenance for planting seems to have been an important factor. Other diebacks of unknown cause have occurred from time to time. However, the critical analysis of the literature, conducted as part of the work of the Concerted Action, has largely disposed of the idea that the Phytophthora disease might have been present long before it was recognised in the UK in 1993. It now seems unlikely to have been in existence for much more than, say, 30 years.

Symptoms of the Phytophthora disease are broadly similar across its geographic range and one particular Phytophthora species – known as the 'alder Phytophthora' – is associated with the vast majority of cases (Streito, Chapter 4). The key symptom is the lesion in the inner bark of the stem, which is often marked externally by the production of a tarry or rusty exudate. In severely affected trees, the foliage is small, sparse and often yellowish. Heavy fruiting commonly occurs. Such trees normally die but there is evidence that some do recover (Gibbs *et al.*, Chapter 6).

Of enormous importance is the definitive evidence that the pathogen is of hybrid origin – the putative parents being *P. cambivora* and a fungus close to *P. fragariae* (Brasier, Chapter 5). Neither of these fungi can attack alder, nor are they considered to be native to Europe, both probably having been introduced by man during the course of trade in plants or plant products. It therefore follows that there is every reason to suppose that the alder Phytophthora owes its origin to man's activities. It may well have originated in a nursery – as appears to be the case with the new hybrid between *P. nicotiana* and *P. cactorum* that has recently been detected as a pathogen of *Primula* in the Netherlands (Man in't Veld *et al.*, 1998). The very wide significance of the issue of hybrid pathogens for international plant health has been emphasised by Brasier (2000). On a more positive note, inoculation studies on a range of woody plants, have not provided any evidence that the pathogen can attack trees other than alder.

The causal fungus is now known to comprise a set of hybrids varying in genetic constitution. In consequence, reference is made to the 'alder Phytophthoras' rather than to the 'alder *Phytophthora*'. There is the widely distributed 'standard type' and a number of so-called 'variants', some of which, on present information, show a restricted distribution. The variants may be genetic breakdown products of the standard types or they may be the products of backcrosses or further hybridisation events. Some of them seem to be less pathogenic than the standard type.

Information on current impact is contained in the chapters by Streito (Chapter 4) and Gibbs *et al.* (Chapter 6). The disease has now been recorded in 11 countries, from Ireland in

the west to Lithuania in the east, and from Sweden in the north to Italy in the south. Within this area, available evidence indicates that its occurrence is widespread but very uneven. Where it does occur, mortality has been as high as 70% in some localities but much lower in others. Some survey plots show a rapid and sustained increase in mortality (for example, some of those in the south of the UK and in northeastern France) while in others (such as in Upper Austria) notable recovery from the disease has occurred. In one place, namely the natural wetland of Die Wieden in the Netherlands, a moderately pathogenic variant of the fungus can be present without causing any damage. The whole question of the influence of the vitality of the host on disease development has been scarcely investigated. This may be a factor in the sudden appearance of disease in two streams in the southern part of the Netherlands where water levels had been raised artificially.

From all this it follows that the potential impact of the disease can be determined only by further detailed studies of particular sites, by maintaining the system of surveys and through appropriate experimentation under controlled conditions. At present little is known about the basic biology of the disease (see Lonsdale, Chapter 7). Once adequate information on the infection process, host invasion and pathogen survival has been obtained, factors determining disease severity will need to be tackled. There may well be processes at work that can prevent the disease from developing in one locality or, by contrast, that can trigger a destructive outbreak in another.

The possibility of limiting pathogen spread

The evidence provided by Gibbs *et al.* (Chapter 6) demonstrates the importance of rivers and other watercourses in the dissemination of the pathogen. It is clear that once the pathogen is established in a river system there is nothing that can be done to prevent downstream dispersal. However, there is also strong evidence that alders from nurseries have played a regrettably large role in the introduction of the pathogen to new areas. In view of the fact that, despite careful investigation, the disease has not been found in European countries such as Finland and Norway (Streito, Chapter 4), partners in the Concerted Action have concluded that there is every reason to seek to minimise further long-distance dissemination of the pathogen on nursery plants. To this end the partners produced the following recommendations at the final workshop in November 2000:

1. Strict phytosanitary regulations should be imposed on plants entering disease-free countries in Europe and elsewhere in the Northern Hemisphere.
2. A certification scheme should be developed for alder plants coming from the nursery once PCR techniques and suitable sampling schemes have been developed. The procedures used for *Phytophthora* detection on strawberry plants will provide a useful precedent.
3. Until a certification scheme is in place, it is recommended that a 'Code of Good Practice for Nurseries' should be developed. *Inter alia* this should ensure that:
   - There is no river irrigation of the nursery.
   - There is a growing season inspection of alder plants.
   - There should be routine disinfection of the nursery between crops; this should include the best available information on chemicals (for the short-term at least), steam sterilisation, rotation and practices such as green manuring.

Options for disease management and control

It is not possible from the work conducted to date to produce a critical evaluation of options for control of the disease in areas where it is

established (see Gibbs, Chapter 8). Studies on the effects of coppicing diseased stools are still at a very early stage and very little is known about the incidence of disease that can be expected in the new growth. Chemical treatments to protect or cure individual trees remain untried. There is a case for critical evaluation of phosphite given its relatively benign nature, but it is unlikely that any spray or injection treatment with this material would be widely used.

So far the studies on host resistance have also given rather disappointing results, indicating that resistance genes may be rare and that their identification may require a fairly long-term screening programme. However, experience with work on resistance in Port Orford cedar to the lethal *Phytophthora lateralis* (see Bower *et al.*, 2000) gives encouragement that this is by no means a hopeless task. The diversity and instability in the causal fungus need to be borne in mind, as does the requirement to produce plants that will grow well in their final location – on the river bank or in a wetland. Genotypes that perform satisfactorily in the nursery will not necessarily be a success in the field!

Future research needs

The great benefits offered by the Concerted Action have already been outlined. At the same time it must be recognised that there have been certain frustrations, most notably through the recognition that research could only develop as the funding of the partner organisations permitted. Some clearly valuable avenues could not be followed up and the gaps in current knowledge are very apparent from a perusal of this text, in particular, the biology of infection and host colonisation. Listed below are the key priorities as determined at the final workshop:

1. Develop molecular tools for the identification of alder Phytophthoras in soil, water and host tissue.
2. Understand the infection process, including survival of the pathogen, and propagule dissemination; this should include a special study of the situations where the alder *Phytophthora* is present without causing disease.
3. Understand factors influencing host invasion, with special reference to the influence of environmental factors on the ability of the host to resist the effects of the pathogen.
4. Extend knowledge of the diversity and stability of the alder Phytophthora genotypes found across Europe.
5. Establish and maintain monitoring plots to enable disease development to be modelled and predicted.
6. Screen for resistance to the pathogen, taking particular note of trees showing freedom from disease in areas of high mortality.
7. Investigate the role of nurseries in dissemination.
8. Develop control strategies in the nurseries.

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Cees van Dijk worked for many years as a senior scientist in the Department of Plant Micro-organism Interactions at the Netherlands Institute of Ecology. Among his many studies, those directly relevant to Phytophthora disease of alder include work on the ecology of *Frankia* in alder and the diversity of Oomycetes in managed vegetation.

Joan Webber is the Principal Pathologist of Forest Research, the Forestry Commission Research Agency. She currently leads FC research on Phytophthora disease of alder, and is also actively involved in other projects including *Phytophthora ramorum* (the cause of Sudden Oak Death), various quarantine plant health issues and the use of biocontrol agents in forestry.

Alders play a vitally important role in Europe. Their diversity of characters not only enables them to establish as pioneers but in many cases also allows them to play a significant part in climax forests and make a major contribution to the ecology and stability of river banks. All four European alder species are important in the establishment of woodland on difficult sites. European alders are largely free from major pest and disease problems so the discovery of a previously unknown disease, caused by a new Phytophthora fungus, led to major Concerted Action within Europe.

Based on the objectives of this Concerted Action, the Bulletin sets out to:

- determine if the spread of the disease within Europe can be limited.
- define the nature of the pathogen.
- make recommendations on disease management and control.
- identify future research requirements.

Although aimed primarily at forest pathologists and managers, it is hoped that these issues will prove to be of interest to a wider audience, coupled with the fact that work on alder Phytophthora disease has also thrown up important information about the generation of new pathogens through unusual hybridisation events and other evolutionary processes.

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