Forest Research Institute

Alien Invasive Species and International Trade

Edited by
Hugh Evans and Tomasz Oszako

Warsaw 2007

Reviewers:
Steve Woodward (University of Aberdeen, School of Biological Sciences, Scotland, UK)
François Lefort (University of Applied Science in Lullier, Switzerland)

ISBN 978-83-87647-64-3

Description of photographs on the covers:
Alder decline in Poland – T. Oszako, Forest Research Institute, Poland
ALB Brighton – Forest Research, UK;
Anoplophora exit hole (example of wood packaging pathway) – R. Burgess, Forestry Commission, UK
Cameraria adult Brussels – P. Roose, Belgium;
Cameraria damage medium view – Forest Research, UK;
other photographs description inside articles – see Belbahri *et al*.

Language Editor: *James Richards*
Layout: *Grażyna Szujecka*

Print: Sowa–Print on Demand www.sowadruk.pl, phone: +48 022 431 81 40

Instytut Badawczy Leśnictwa
05-090 Raszyn, ul. Braci Leśnej 3, phone [+48 22] 715 06 16
e-mail: Wydawnictwa_IBL@ibles.waw.pl

CONTENTS

Part I – EXTENDED ABSTRACTS

INTRODUCTION

This book summarises the presentations at the Inaugural meeting IUFRO Unit 7.03.12 "Alien Invasive Species and International Trade" which was held in Poland, 3-7 July, 2006 in the Ecological Education and European Integration Centre of State Forests, in Jedlnia.

The meeting was attended by over 60 delegates from 18 countries representing both the northern and southern hemispheres. One of the purposes of this new IUFRO Unit is to encourage cross-disciplinary exchange in relation to all types of Alien Invasive Species (AIS) and, to this end, the programme included consideration of a wide range of biotic agents, principally invertebrates and tree pathogens, that could be classified as AIS.

As presented in this book, the papers are organised into four main topics:

- Alien invasive species in Europe,
- World ecological impact,
- Detection and policy,
- Prevention measures and treatments.

There is a broad range of scientific topics presented and discussed ranging from the damage caused by *Phytophthora* species, through analysis of pathways for international movement of pests and pathogens, to the implications of scientific findings for policy and regulatory frameworks.

The content of the book presents both scientific and regulatory papers, reflecting the range of expertise of the delegates. This provides a forum for exploration of issues arising from research and from experiences in managing the regulation of pathways to reduce or prevent international movement of AIS. There are topics of universal interest and valuable opinions in relation to pathways for international movement of AIS:

Wood packaging material and implementation of ISPM15*

This is very much a current topic and several papers address this issue. The main concerns are whether residual bark on ISPM15 treated wood pose a threat through infestation after treatment and, secondly, how reliable the ISPM15 mark is in indicating that treatment had actually been carried out. There is an increasing body of research that provides evidence for successful breeding by bark and wood boring beetles on wood with bark that has been subjected to ISPM15 treatments, whether by heat treatment or methyl bromide fumigation. Papers by Robert Haack (USA) and by Hugh Evans (UK) confirm this finding and, in addition, both authors consider the significance of the amount of residual bark in quantifying the risks. The conclusions from their experimental studies and from survey work of packaging wood in trade carried out by Robert Haack in the USA was that bark pieces approximately greater than a credit card in size (i.e. approximately 45 cm^2) had associated live insects that could enable bark beetle species to complete their life cycles. Hugh Evans raises the question of how many beetles emerging actually constitute a successful founder population. Discussion on different types of packaging wood, particularly the distinction between

---

*ISPM15 – International Standards on Phytosanitary Regulations No 15: Guidelines for regulating wood packaging material in international trade manufactured packaging such as pallets (discussed by Charles Ray (USA)) and dunnage, concentrate on the amount of residual bark that could be present. It is clear that packaging wood such as pallets are produced to industry standards that, irrespective of phytosanitary regulations, require a high degree of freedom from bark. This inevitably leads to discussion on how to define freedom from bark (i.e. debarked *vs* bark-free) and the need for more rigorous definitions of bark freedom. Overall, it is felt that dunnage, usually produced from salvage wood, represents the highest risk because it tends to have more associated bark and is also more difficult to treat to ISPM15 standards.

The question of how reliable the ISPM15 mark is in providing assurance that the wood has actually been successfully treated is raised by Shiroma Sathyapala (NZ) who points out that there is a need for confidence in the system and that sharing of interception data, particularly for instances of non-compliance, will be a step forward. Eric Allen (Canada) points out that NAPPO* shares lists of the top 10 countries with lowest compliance. It is agreed that it will be valuable to categorise the pathways within the broader definition of packaging wood and to consider the risks from treatment failure and/or presence of residual bark for each pathway. Bill Aley (USA) and Jon Sweeney (Canada) suggest that further surveillance of wood packaging, including pallets and other categories, with the ISPM15 mark will yield the data necessary to categorise pathways and their associated risks.

Further discussion is needed for alternative treatment regimes within ISPM15, particularly for countries where current methods are difficult to implement, because of the costs and lack of proper infrastructure issues necessary in establishing compliance systems. Clement Chilima (Malawi) indicates that expertise in capacity building will be valuable and that public awareness of the issue is poor in developing countries. He also feels that aspects such as natural resistance to pests for certain tree species could be used to categorise them for ISPM15 compliance. Jacques Gagnon (Canada) feels that drawing together all available information on current options, particularly categorised according to different types of wood packaging will help to identify both risks and possible treatment options. Barbara Illman (USA) suggests that industry tends to solve problems according to the need and that training and demonstration of technologies will help to resolve technical issues.

Plants for planting as a pathway for movement of pests and pathogens

Plants for planting as a pathway for invasive organisms is a topic of concern being consistently cited as high risk, particularly for invasive pathogens. It is acknowledged that phytosanitary rules are already in place in relation to named organisms and that full compliance with these rules should provide acceptable protection. However, it is also acknowledged that (a) inspection-based regimes are unlikely to pick up all infringements especially with large increases in trade, (b) that an organism-based process will tend to miss new and emerging pest and pathogen problems and (c) that application of treatment methods to remove invasive organisms is more difficult for this pathway.

In general, delegates felt that analysis of the ‘plants for planting’ pathway to identify ‘keystone’ damaging organisms would enable construction of a database of biological characteristics of AIS that are most likely to exploit this pathway for transportation to new loca-

---

*North American Plant Protection Organisation tions. It is felt that such a biologically-based list will be of more value than the current named organism approach and will enable analysis of species lists in exporting countries to assess likelihood of association with live plants for export. During a wide-ranging discussion, it was agreed to establish a sub-group, under the leadership of Kerry Britton (USA), to analyse which nursery stock 'pests' (to include invertebrates and pathogens) have become problems in forestry systems is the issue of a great importance and to provide a position paper on this important issue. The NAPPO Plants for Planting standard (which is based on clean stock programs) and IPPC are also moving towards this basis for the Plants for Planting pathway. Sharing information and experiences in early detection of pests and pathogens on pathways, including plants for planting is of a crucial significance to reach the goal of reducing the risks of movements of pests along this pathway.

Furthermore, live plants are the subject of consideration of risks of weediness in relation to the plants themselves becoming 'pests'. Characteristics of weediness have already been discussed broadly in the scientific literature. There is much to be learned from knowledge of adaptation of local pest and pathogen species to exotic trees and shrubs as a further element in the risk profile of plants for planting.

The papers indicate that 'Plants for Planting' is a pathway that requires greater attention and will be a core item for the Unit as it develops and prepares for future meetings.

The book provides a focus for forest 'pests' and their movement internationally, but it is clear from papers by Alain Roques (France), Wojciech Solarz (Poland), Clement Chilima (Malawi) and Stas Burgiel (USA) that there are many initiatives, nationally and internationally, in this area. There is a clear need to maintain good communication and coordination between the various initiatives and, at least for the forestry sector, Unit 7.03.12 could provide a 'one-stop shop' for linkage.

Dr. Hugh Evans and Dr. Tomasz Oszako

PART I

EXTENDED ABSTRACTS

PHYTOPHTHORA ROOT AND COLLAR ROT OF ALDERS CAUSED BY THE INVASIVE *PHYTOPHTHORA ALNI*: ACTUAL DISTRIBUTION, PATHWAYS, AND MODELED POTENTIAL DISTRIBUTION IN BAVARIA

Thomas Jung[1], Marla Downing[2], Markus Blaschke[3], Thomas Vernon[2]

[1]Independent Scientist and Consultant for Tree Diseases, Thomastrasse 75, D-83098 Brannenburg, Germany, www.tree-diseases.com, competence@tree-diseases.com.
[2]Forest Health Technology Enterprise Team, Forest Health Protection, USDA Forest Service, Suite 331, 2150 Centre Avenue, Building A, Fort Collins, CO 80526-1891.
[3]Bavarian State Institute of Forestry (LWF), Section Forest Ecology and Forest Protection, Am Hochanger 11, D-85354 Freising, Germany.

HISTORY AND SYMPTOMS OF *PHYTOPHTHORA* ROOT AND COLLAR ROT OF ALDERS

In 1993 a previously unknown lethal root and collar rot disease of common alder (*Alnus glutinosa*) was recorded in southern Britain. It occurred mainly along riverbanks, but also in orchard shelterbelts and woodland plantations (Gibbs *et al.* 1999). In the following years the disease was also found on grey alder (*A. incana*) and Italian alder (*A. cordata*), and in Germany, France, Ireland, Sweden, the Netherlands, Belgium, Austria, Hungary, Lithuania, Poland, Slovenia and Italy (Hartmann 1995; Gibbs *et al.* 1999, 2003; Jung & Blaschke 2003, 2004, 2006; Oszako 2005, Szabó *et al.* 2000; Streito *et al.* 2002; Brasier & Jung 2003). The causal organism was identified as a swarm of interspecific hybrids between *Phytophthora cambivora* and an unknown *Phytophthora* related to *P. fragariae* (Brasier *et al.* 1995, 1999), and recently described as a new species, *Phytophthora alni* sp. nov., which comprises 3 subspecies (Brasier *et al.* 2004).

Infected trees show abnormally small, sparse and often yellowish foliage, a dieback of the crown, early and often excessive fructification with unusually small cones, and tongue-shaped necroses of the inner bark and the cambium which extended up to 3 m from the stem base with tarry or rusty spots on the surface of the bark (Jung & Blaschke 2001; Figs. 1–4).

Figure 1. Grey alder (*Alnus incana*) growing in a plantation on former agricultural land with sparse, chlorotic and small-sized foliage due to *Phytophthora alni* root and collar rot

Figure 2. Mature, riparian common alder (*Alnus glutinosa*) stand with high impact of *P. alni* root and collar rot

Figure 3. Grey alder (*A. incana*) with collar rot caused by *P. alni;* typical tarry spots at the outer bark and tongue-shaped orange-brown necrosis of the inner bark

Figure 4. Mature common alder (*A. glutinosa*) with collar rot by *P. alni* (tarry spots at the outer bark) growing in a forest stand; introduction of the pathogen via infested nursery stock used for the establishment of the young alder plantation visible in the background

ACTUAL DISTRIBUTION OF *P. ALNI* IN RIPARIAN AND FOREST ECOSYSTEMS IN BAVARIA, AND POTENTIAL PATHWAYS

In Bavaria, Southern Germany, a detailed survey of *Phytophthora* root and collar rot of common and grey alder was performed in 2001 and 2002 by the Bavarian State Forestry and the river authorities.

The disease was found in 1041 out of 3247 surveyed forest alder stands (32.1%) (Fig. 5). The majority of the affected stands (80.9%) were less than 21 years old (Fig. 6). Almost half of these young stands (46%) were growing on non-flooded sites and the majority (92%) were planted, strongly suggesting the introduction of the pathogen with infected nursery stock.

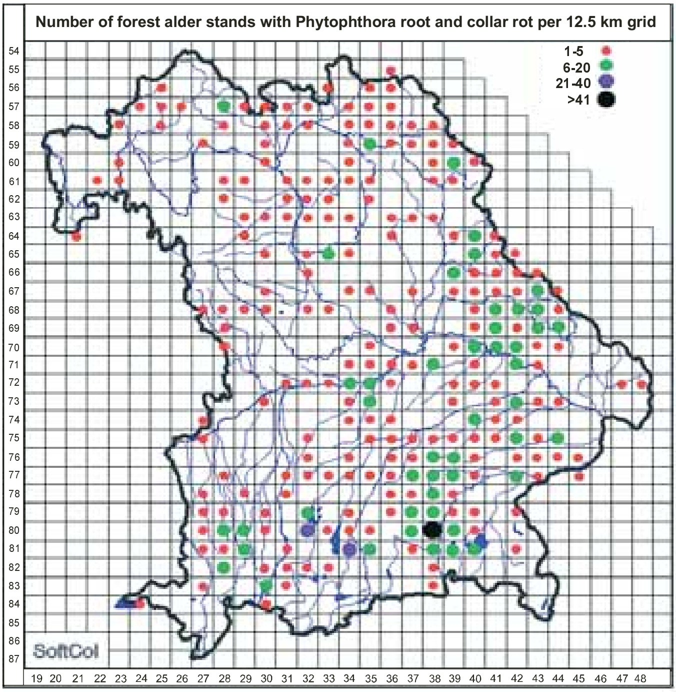


Figure 5. Distribution of forest stands of alder suffering from *Phytophthora* root and collar rot in Bavaria

The riparian survey demonstrated that the disease is widespread along more than 50% of the river systems (Fig. 7). Along some rivers the disease incidence exceeds 50%.

A small-scale nursery survey demonstrated presence of *P. alni* in rootstocks of alder plants from three out of four Bavarian nurseries which regularly bought alder plants from intensive, large-scale commercial nurseries for resale. The rootstocks from four nurseries that grew their own alder plants from seeds were not infested by *P. alni* (Tab. 1; Jung & Blaschke 2003, 2004). At least two of the three infested nurseries used irrigation water taken from rivers with diseased alder stands upstream from which *P.*

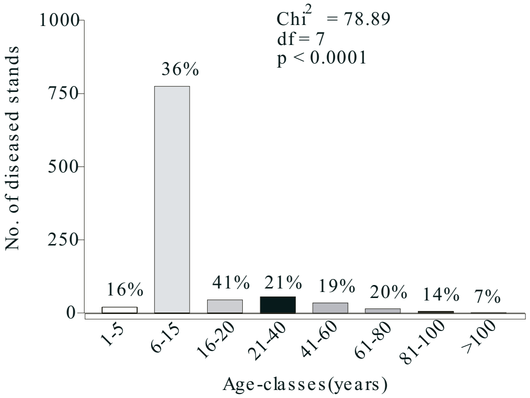


Figure 6. Number and proportion of diseased forest alder stands in different age classes in Bavaria

alni was recovered. Interestingly, the alder plants from the infested nursery beds looked visibly healthy. However, 2 weeks after a second flooding period in the greenhouse bark necroses developed in the root system and at the collar from which *P. alni* was isolated. This demonstrates the infeasibility of current control protocols of nursery stock. Moreover, six other *Phytophthora* species were also baited from the alder plants, among them *P. cactorum*, *P. cambivora*, *P. citricola* and *P. quercina*, all involved in current widespread declines of deciduous trees and forests across Europe (Jung 2006; Jung *et al.* 1999, 2000, 2002, 2003, 2005; Brasier & Jung 2003, 2006; Hartmann *et al.*

Figure 7. Distribution of *Phytophthora* root and collar rot of alders along main rivers and streams in Bavaria; small map showing the location of Bavaria within Germany

Table 1. Isolation results from nursery-grown plants in Bavaria

Nursery no.	Alder species [a]	*Phytophthora* species[b]								
		ALN	ALN×CAM	CAM	MEG	CAC	CIT	GON	QUE	CHLA
Nurseries buying in alder plants for resale										
1	GLU	X[c]	–	–	–	–	–	–	–	–
2	GLU	–	X	X	X	–	X	X	–	X
	INC	X[d]	–	X	–	–	X	X	–	X
	VIR	–	–	X	–	–	X	–	–	–
3	GLU	–	–	–	X	–	X	X	–	X
4	GLU	X[c]	–	X	–	–	–	X	–	X
Nurseries growing alder plants from seeds										
5	GLU	–	–	–	X	X	X	–	–	–
6	GLU	–	–	–	–	–	–	–	X	–
7	GLU	–	–	X	X	X	X	–	–	–
8	GLU	–	–	–	–	X	X	–	–	–

[a] GLU = *Alnus glutinosa*, INC = *A. incana*, VIR = *A. viridis*.

[b] ALN = *P. alni*, ALN x CAM = putative backcrosses between *P. alni* and *P. cambivora* (according to ITS data), CAM = *P. cambivora*, MEG = *P. megasperma*, CAC = *P. cactorum*, CIT = *P. citricola*, GON = *P. gonapodyides*, QUE = *P. quercina*, CHLA = 'P. taxon Pgchlamydo', a yet undescribed chlamydospore forming species close to *P. gonapodyides* (Brasier & Jung, 2003), X = isolated, – = not isolated.

[c] *P. alni* ssp. *alni*.

[d] *P. alni* ssp. *alni* and *P. alni* ssp. *uniformis*

2006; Jönsson *et al.* 2005; Balci und Halmschlager 2003; Vettraino *et al.* 2001, 2002). In another study in Brandenburg Schumacher *et al.* (2005) recovered *P. alni* from alder fields of 5 out of 9 nurseries. It has to be noted that *P. alni* may also be transported passively with the trade of bare rooted nursery stock as it is able to adhere to the fine roots of non-host tree species exposed to the pathogen.

An intensive quest for the source of inoculum in 60 infested river systems (Tab. 2) demonstrated that the introduction of *P. alni* into a river system occurs primarily via the

Table 2. Contingency table occurrence of *P. alni* infested plantation in catchment area /*Phytophthora* root and collar rot of riparian alders

	Number of rivers with diseased alders	Number of rivers with healthy alders	Total
Infested plantation in catchment area present	58	0	58
Infested plantation in catchment area absent	2	25	27
Total	60	25	85
Relative risk = 13.5; 95% - CI: 3.556 – 51.24; Fishers exact test: p <0.0001			

planting of infested nursery stock on the river banks or on forest sites that drain into the rivers (Jung & Blaschke, 2003, 2004). In two rivers, diseased natural alder stands were only found downstream and on the banks of the raceways of commercial fish farms with international trade. Most probably the introduction of *P. alni* occurred with basin water from other fish farms whose raceways are fed by infested river water.

Once introduced to a river system, *P. alni* spreads downstream infecting the collar or bare roots of riparian alders via lenticels and adventitious roots.

The wide distribution of the disease in Bavaria is due to the unintended use of infested alder stock for the stabilisation of steep slopes and banks of whitewater rivers, initial afforestations of former agricultural land, and in woodlands on wet sites (Jung & Blaschke 2003, 2004).

For the first time in history it could be shown clearly that a devastating epidemic by an invasive pathogen was caused by the spread of infested nursery stock.

As temporary control measures coppicing of infected alder trees and stools is recommended along water courses (Gibbs 2003) but not in infested forest plantations (Jung & Blaschke 2006). A code of good practice was developed for Bavarian forest nurseries; major tasks were (i) the production of alder plants from seeds on fields that were free from any tree growth for at least 3 years or in containers with steam sterilized soil and (ii) the abandonment of river and surface water as irrigation water (Jung & Blaschke 2004, 2006). Some survivors in highly infested common alder stands were shown to be less susceptible to *P. alni* than declining trees, and on the longterm a resistance screening program may help to sustain alders as major components of riparian and swamp forests (Jung & Blaschke 2006).

MODELLING THE POTENTIAL DISTRIBUTION OF *P. ALNI* IN BAVARIA

Using a binary classification tree, GIS, field sample data, and coarse-resolution auxiliary data, a model was developed for predicting the potential distribution of *P. alni* (PDPA) in Bavaria. We only investigated forested lands in Bavaria due to budget constraints.

A total of 307 *P. alni* infested and 127 non-infested alder tree locations were identified in forested areas in Bavaria between the spring of 2003 and the winter of 2006. Among the 307 infested sample points, there were 232 points where alder trees had been planted, and 75 points where alders were naturally occurring. Of the 127 healthy non-infested sample points, 38 were planted and 89 had natural alder growth. The 434 sample locations served as the dependent variable dataset in the classification tree analysis. Independent variables included twelve 93 meter datasets on physiography and vegetative cover. These consisted of soil texture components (minimum, mean and maximum percentage values for sand, silt and clay on polygons), aspect, slope, and landform as well as a Normalized Difference Vegetation Index (NDVI) calculated from the Moderate Resolution Imaging Spectroradiometer (MODIS) satellite imagery at 250 meters.

The classification tree identified five independent variables important in predicting the presence or absence of *P. alni*, ie. silt minimum values less than 20% (range = 0 to 80%), sand mean values less than 5%, slope less than 2.97 degrees (range

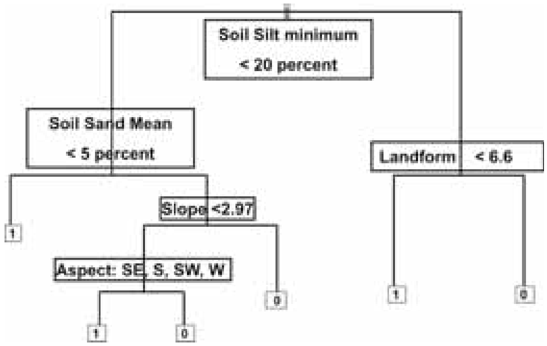


Figure 8. *Phytophthora alni* classification tree for forested areas in Bavaria

= 0 to 30.74 degrees), aspects that were Southeast, South, Southwest, and West, and landform (a measure of concavity and convexity) less than 6.6 (range = -15.20 to +21.60; <0 = concave; 0 = totally flat; >0 = convex) (Fig. 8). Where these ecological factors occur together in the environment, the likelihood of infection is increased.

These factors make biological sense. With decreasing silt and sand values the clay content of the site is increasing. Clay rich sites and sites with fairly flat or concave physical structure have poor drainage thus providing optimum conditions for *P. alni* to produce sporangia and spread and infect via zoospores. Likewise, sites with warmer aspects favour the growth of *P. alni* and thus the invasion of alder bark after infection.

A ten-fold cross validation, used to test the accuracy of the PDPA model, had an error rate of 0.1751 (78.34% model accuracy). *P. alni* infested sample points were predicted with 86% accuracy, and non-infested sample points with 63% accuracy. The higher accuracy for predicting the *P. alni* infested sites compared with predictions for

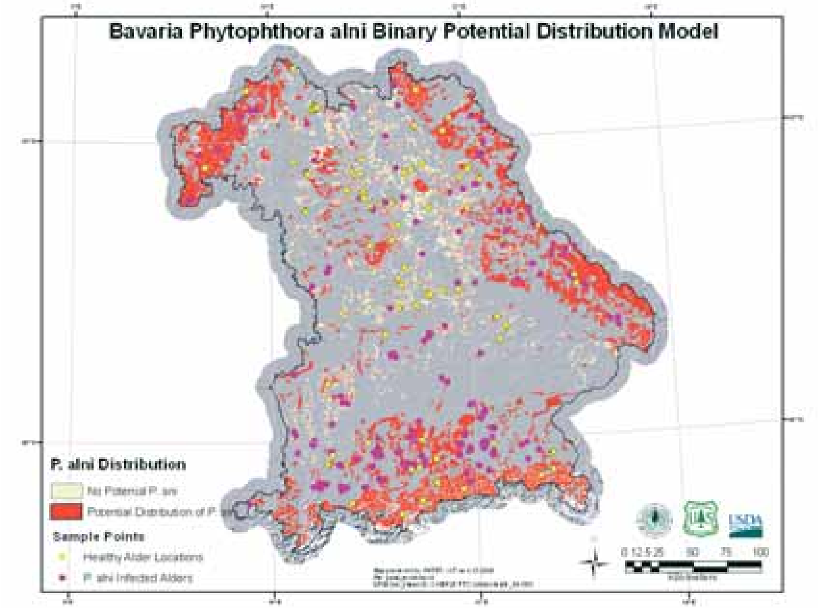


Figure 9. Location of 307 *P. alni* infested and 127 non-infested alder stands in Bavaria, and potential distribution of *P. alni* according to the binary *P. alni* potential distribution model

non-infested sites was most probably due to having three times more infested than non-infested sample locations.

Of the 1.962 million hectares of forests in Bavaria, approximately 1.4015 million hectares (71.43%) were modeled to have a high potential for *P. alni* root and collar rot, 0.5604 million hectares (28.56%) were modeled to have a high potential to remain healthy (Fig. 9).

Literature

Balci Y., Halmschlager E. 2003. Incidence of *Phytophthora* species in oak forests in Austria and their possible involvement in oak decline. Forest Pathology, 33: 157-174.

Brasier C. M., Jung T. 2003. Progress in understanding *Phytophthora* diseases of trees in Europe. [In:] *Phytophthora* in forests and natural ecosystems (eds. McComb J. A., Hardy G. E. St. J., Tommerup I.). Proceedings of the Second International Meeting of IUFRO Working Party 7.02.09, Albany, Western Australia, 2001, Murdoch University, Perth, Australia, 4-18.

Brasier C. M., Jung T. 2006. Recent developments in *Phytophthora* diseases of trees and natural ecosystems in Europe. [In:] *Phytophthora* in Forests and Natural Ecosystems (eds C. M. Brasier, J. Webber, T. Jung). Proceedings of the 3rd International IUFRO Working Party 7.02.09 Meeting, 11th–17th Sept. 2004, Freising, Germany. Forestry Commission, Edinburgh, UK, in press.

Brasier C. M., Rose J., Gibbs J. N. 1995. An unusual *Phytophthora* associated with widespread alder mortality in Britain. Plant Pathology, 44: 999-1007.

Brasier C. M., Cooke D. E. L., Duncan J. M. 1999. Origins of a new *Phytophthora* pathogen through interspecific hybridisation. Proceedings of the National Academy of Sciences, USA 96, 5878–5883.

Brasier C. M., Kirk S. A., Delcan J., Cooke D. E. L., Jung T., Man in't Veld WA, 2004. *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. Mycological Research, 108: 1172-1184.

Gibbs J. N., Lipscombe M. A., Peace A. J. 1999. The impact of *Phytophthora* disease on riparian populations of common alder *Alnus glutinosa*) in southern Britain. European Journal of Forest Pathology, 29: 39-50.

Gibbs J. N., Van Dijk C., Webber J. F. (eds) 2003. *Phytophthora* disease of alder in Europe. Forestry Commission Bulletin 126, Edinburgh, UK, pp 82.

Hartmann G. 1995. Wurzelhalsfäule der Schwarzerle (*Alnus glutinosa*) – eine bisher unbekannte Pilzkrankheit durch *Phytophthora cambivora*. Forst und Holz, 50: 555-557.

Hartmann G., Blank R., Kunca A. 2006. Collar rot of *Fagus sylvatica* caused by *Phytophthora cambivora* – damage, site relations and susceptibility of broad leaf hosts. [In:] *Phytophthora* in Forests and Natural Ecosystems (eds C. M. Brasier, J. Webber, T. Jung). Proceedings of the 3rd International IUFRO Working Party 7.02.09 Meeting, 11th –17th Sept. 2004, Freising, Germany. Forestry Commission, Edinburgh, UK, in press.

Jönsson U., Jung T., Sonesson K., Rosengren U. 2005. Relationships between health of *Quercus robur*, occurrence of *Phytophthora* species and site conditions in southern Sweden. Plant Pathology, 54: 502-511.

Jung T. 2006. Root and collar rot and aerial bleeding cankers of beech in Bavaria caused by *Phytophthora* spp. [In:] *Phytophthora* in Forests and Natural Ecosystems (eds C. M. Brasier, J. Webber, T. Jung). Proceedings of the 3rd International IUFRO Working Party 7.02.09 Meeting, 11th –17th Sept. 2004, Freising, Germany. Forestry Commission, Edinburgh, UK, in press.

Jung T., Blaschke M. 2001. *Phytophthora* –Wurzelhalsfäule der Erlen (*Phytophthora* collar rot of alders). Bavarian State Institute of Forestry (LWF), Freising, Germany, LWF Merkblatt (LWF leaflet) No. 6.

Jung T., Blaschke M. 2004. *Phytophthora* root and collar rot of alders in Bavaria: distribution, modes of spread, and possible management strategies. Plant Pathology, 53: 197-208.

Jung T, Blaschke M. 2006. *Phytophthora* dieback of alders in Bavaria: distribution, pathways and management strategies. [In:] *Phytophthora* in Forests and Natural Ecosystems (eds C. M. Brasier, J. Webber, T. Jung). Proceedings of the 3rd International IUFRO Working Party 7.02.09 Meeting, 11th –17th Sept. 2004, Freising, Germany. Forestry Commission, Edinburgh, UK, in press.

Jung T., Blaschke H., Oßwald W. 2000. Involvement of *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. Plant Pathology, 49: 706-718.

Jung T., Cooke D. E. L., Blaschke H., Duncan J. M., Osswald W. 1999. *Phytophthora quercina* sp. nov., causing root rot of European oaks. Mycological Research, 103: 785-798.

Jung T., Hansen E. M., Winton L., Oßwald W., Delatour C. 2002. Three new species of *Phytophthora* from European oak forests. Mycological Research, 106: 397-411.

Jung T., Nechwatal J., Cooke D. E. L., Hartmann G., Blaschke M., Oßwald W. F., Duncan J. M., Delatour C. 2003. *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. Mycological Research, 107: 772-789.

Jung T., Hudler G. W., Jensen-Tracy S. L., Griffiths H. M., Fleischmann F., Oßwald W. 2005. Involvement of *Phytophthora* species in the decline of European beech in Europe and the USA. Mycologist, 19: 159-166.

Oszako T. 2005. Zamieranie olszy w Europie. Leśne Prace Badawcze., suplement 1: 53-63.

Schumacher J., Leonhard S., Grundmann B. M., Roloff A. 2005. Neuartiges Erlensterben im Biosphärenreservat Spreewald – Ursachen, Verbreitung, Folgen und Gegenmaßnahmen (novel alder mortality in the biosphere reserve Spreewald – causes, distribution, consequences and management). Dendro-Institute Tharandt (ed.), Technical University Dresden, 1-43.

Streito J. C., Legrand P., Tabary F., Jarnouen de Villartay G. 2002. *Phytophthora* disease of alder (*Alnus glutinosa*) in France: investigations between 1995 and 1999. Forest Pathology, 32: 179-191.

Szabó I., Nagy Z., Bakonyi J., Érsek T. 2000. First report of *Phytophthora* root and collar rot of alder in Hungary. Plant Disease, 84: 1251.

Vettraino A. M., Natili G., Anselmi N., Vanini A. 2001. Recovery and pathogenicity of *Phytophthora* species associated with resurgence of ink disease on *Castanea sativa* in Italy. Plant Pathology, 50: 90-96.

Vettraino A. M., Barzanti G. P., Bianco M. C., Ragazzi A., Capretti P., Paoletti E., Luisi N., Anselmi N., Vannini A. 2002. Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. Forest Pathology, 32: 19-28.

STUDIES ON THE OCCURRENCE OF *PHYTOPHTHORA RAMORUM* IN NURSERIES, FOREST STANDS AND GARDEN CENTERS

Tomasz Oszako[2], Leszek B. Orlikowski[1], Aleksandra Trzewik[1],
Teresa Orlikowska[1]

[1]Institute of Pomology and Floriculture in Skierniewice; e-mail: lorlikow@insad.pl
[2] Forest Research Institute, Department of Forest Phytopathology, Sękocin,
05-090 Raszyn, Poland; e-mail: T.Oszako@ibles.waw.pl

INTRODUCTION

The aim of the work described here has been to determine the interrelationships between host plants of *Phytophthora ramorum*, the place and time of the screening and collection of samples of diseased plants for study on the one hand, and the frequency of occurrence of this pathogen and other *Phytophthora* species in infected tissues on the other. The colonization of the tissues of different species of plant by isolates of *P. ramorum* was also assessed. In the below paper the interdependence between host plants for *P. ramorum*, the place and time of screening and of the collection of samples of sick plants for study, and the frequency of occurrence of the pathogen and other phytophthora species in infected tissues in discussed.

MATHERIAL AND METHODS

The work commenced on March 15th, ending in mid-November 2006. The subjects of interest were the following plants, as potential hosts of *P. ramorum*: rhododendron, pieris, heathers and heaths, cowberry, mountain laurel and lilac (Table 1). Stands of beech and red oak in 4 forests were also put under observation. The most frequent subjects of analysis were different varieties of rhododendron, among them Blurettia, Blutopia, Catawbiense Grandiflorum, Cunningham White, Erato, Excelsion, Fantastica, Goldbukett, Hachmanns Charmant, Lachsgold, Mieszko, Nova Zembla, Polarnacht, Pohjola's Daughter, Pumuckl, Silberwolke, Slippy and Torero. The analysed varieties of pieris were in turn Cupido, Heath G., Flamingo Silver, Forest Flame, Mountain Faire and Variegata. There were 16 varieties of heather and heath, but none of them yielded isolates of *Phytophthora*. These plants were observed, and

---

The research was supported by the State Committee for Scientific Research (KBN, Poland)
grant no 2P06L 041 28

Table 1. Compilation of data on afflicted plant material and the number of places from which it was collected

Detail	*Calluna vulgaris*	*Pieris japonica*	*Rhododendron* spp.	*Kalmia auqustifolia*	*Syringa vulgaris*	*Vaccinium vitis-idaea*
No. of plants studied	58	73	143	11	45	28
No. of varieties studied	5	6	18	1	3	1
Number of places from which plants werecollected	12	15	43	6	1	5

sampled from, in 5 in 8 forests, in 8 allotments and at 22 garden centres (Table 1). The screening of plants in these places was done once (in the forests and allotments), 2–5 times at the garden centres, and at least 15 times in the 4 nurseries. The fact that screening was carried out so often at the nurseries was connected with the large numbers of plants produced there, as well as a desire to uncover the most important source of *P. ramorum*, i.e. nursery material.

The plants studied were mainly of the family *Ericaceae*, showing symptoms of shoot-apical dieback, necrosis spreading from stems on to leaf-blades or patches on leaves. An interest was also taken in leaf-blades that had fallen off plants, where the aforementioned symptoms were present on them. Shoots displaying symptoms of disease were cut several centimeters below the browning area, and placed in plastic bags identified by place and date of sampling, variety name and where possible section number. They were then transferred to the laboratory. Samples were generally analysed the following day, having been maintained overnight at a temperature of around 5ºC. Infected leaves were separated from stems with symptoms of tissue-browning, washed carefully under running water, and then rinsed 3 times in distilled water. The material prepared in this way was dried between two layers of sterile filter paper. The remainder of the preparation procedure was as detailed in Orlikowski and Szkuta (2002). The fraction of leaves that had fallen off plants was also placed into the soil extract, and incubated for 48 hours at a temperatures in the range 20–22ºC, before fragments of tissue were observed under a microscope for the presence of *P. ramorum*. Dishes with fragments of infected leaf or stem tissue were reviewed during the 96 hours of their incubation, any growing colonies of fungi or phycomycetes being transferred on slants with PDA. The fungal cultures obtained were identified to genera and species levels using relevant keys and monographs.

Isolates of *Phytophthora* were grouped on the basis of the appearance of colonies and thalli under the microscope. Representative cultures were identified to species level on the basis of morphological features, as well as by means of molecular methods. Isolates for identification were preserved on slants and on Petri dishes in potato-glucose agar (PDA), V8 agar and Organic Medium. The isolation of nucleic acid from pure cultures made use of the method devised by Aljanabi and Martinez (1997), as modified by substitution of the grinding of mycelium in a homogenizer or by using liquid nitrogen, with normal crushing of mycelium with remains of medium.

Identification of species on the basis of the DNA isolated from pure cultures was achieved using the RAPD: C85, C92 starters (Lee *et al.*, 1996), as well as starters ISSR:

808, 827, 889 and 890 (UBC series 800) and AL4 (Lisek, 2002). Band paterns of the studied isolates were compared with those for 14 known species of *Phytophthora* (reference isolates for *P. alni, P. cactorum, P. cambivora, P. cinnamomi, P. citricola, P. citrophthora, P. cryptogea, P. nicotianae var. nicotianae, P. palmivora* and *P. ramorum*). Reaction conditions (the thermal profile and composition of the reaction mixture) had been selected empirically in 2002 (Wiejacha *et al.* 2002). The products of PCR were separated on 1.4% agarose gels (Sigma-Aldrich) at a voltage of c. 4V per cm of gel and with ethidium bromide staining.

The identification of *P. cactorum*, *P. cambivora*, *P. citricola*, *P. cryptogea*, *P. citrophthora* and *P. ramorum* made use of starters based on differences in the sequences of ITS (Internal Transcribed Spacer) regions of rDNA, or else – in the case of *P. alni* – of starters whose design was based on SCAR (Sequence-Characterized Amplification Regions) markers (Loos *et al.* 2005, Schubert *et al.* 1999, Ersek *et al.* 1994, Boersma *et al.* 2000.

RESULTS

Isolations of pathogens from infected plants related to the place

Infected shoot tissue yielded no fewer than around 20 genera and species of fungi and Alga like Oomycetes (Table 2). Prevalent among them were *Alternaria alternata, Botrytis cinerea, Fusarium* spp., *Pestalotia* and *Phytophthora citricola* (Table 2) The greatest diversity of microorganisms was reported on rhododendrons and pieris, the most limited on mountain laurel and lilac (Table 2). The isolations of microorganisms from rhododendrons, pieris and heathers were always accompanied by *Pestalotia sydowiana* and *Botrytis cinerea*. In order to identify the 529 isolates obtained, the PCR reaction with non-specific starters (RAPD, ISSR) was run, with 14 reference isolates. Comparison of DNA band profiles of the studied isolates with those from known reference cultures allowed for the preliminary identification of 115 isolates of *P. citricola* and 16 of *P. citrophthora.* The presence of these species was then confirmed by way of PCR with starters specific to the aforementioned pathogens. Analysis of the occurrence of *Phytophthora* species also showed that *P. citricola* and *P. citrophthora* colonised rhododendrons in nurseries 2 and 4, as well as pieris at nursery no. 1. The infected shoots of cowberry, collected from nursery no. 2 yielded *P. citricola*. The next two nurseries (growing heathers) failed to provide any sign of the presence of *Phytophthora* spp. Likewise, heather collected from the 8 forests did not give rise to any *Phytophthora* isolates either. All that could be established for analysed diseased plants at the garden centres and on the allotments was the sporadic presence of *P. citricola* (Table 3).

Summer and autumn 2006 were not, however, favourable to the development of *P. ramorum*, particularly where the forests (and to a great extent also the allotments and garden centres) were concerned. The high air temperature in summer and only sporadic rainfall drastically limited the growth of the species, as well as the formation of zoosporangia and chlamydospores thereby. While it is true that the autumn temperatures were lower, the ongoing lack of rainfall continued to obstruct

Table 2. List of species of fungi and Alga like Oomycetes isolated from infected organs of plants

Calluna vulgaris (shoot apices)	*Kalmia auqustifolia* (stems, leaves)	*Pieris japonica* (stems, leaves)	*Rhododendron* spp. (stems, leaves)	*Vaccinium vitis-idaea* (stems, leaves)	*Syringa vulgaris* (leaves)
Alternaria alternate	*Alternaria alternata*	*Alternaria alternata*	*Acremonium* sp.	*Alternaria alternata*	*Botrytis cinerea*
Botrytis cinerea	*Botrytis cinerea*	*Botrytis cinerea*	*Alternaria alternata*	*Botrytis cinerea*	*Cladosporium* sp.
Fusarium avenaceum	*Fusarium avenaceum*	*Cladosporium* sp.	*Botritis cinerea*	*Mucor* spp.	*Fusarium avenaceum*
Fusarium poae	*Mucor* spp.	*Fusarium avenaceum*	*Cladosporium* sp.	*Phytophthora citricola*	*Mucor spp.*
Mucor spp.		*Fusarium culmorum*	*Fusarium avenaceum*		*Phytophthora citrophthora*
Pestalotia sydowiana		*Mucor* spp.	*F. culmorum*		
Rhizoctonia solani		*Pestalotia sydowiana*	*F. equiseti*		
Trichoderma spp.		*Phytophthora citricola*	*F. poae*		
		Phytophthora citrophthora	*Gliocladium roseum*		
			Mucor spp.		
		Penicillium spp.	*Penicillium* spp.		
			Pestalotia sydowiana		
			Phytophthora citricola		
			Trichoderma spp.		

Table 3. Relationship between place of collection of diseased plants and the isolation of *Phytophthora* spp.

Places from which sick plants were taken	Isolated *Phytophthora* species	
	P. citricola	*P. citrophthora*
Nursery no. 1	+	+
Nursery no. 2	+	+
Nursery no. 3	+	–
Nursery no. 4	–	–
Nursery no. 5	–	–
Garden centres (22)	+	–
Allotments (8)	+	–
Forests (8)	–	–

Table 4. Frequency with which *Phytophthora* spp. were isolated from infected pieris and rhododendron plants in 2006; number of plants from which species of this genus were isolated

Phytophthora species	*Pieris japonica* Total of 73 plants	*Rhododendron* spp. Total of 143 plants
P. citricola	17	90
P. citrophthora	21	–

development or the pathogen and the colonisation of host plants. In turn, in the nurseries, the sprinkling of plants often afforded good humidity conditions for the development of *P. ramorum*. However, the factor limiting growth and spore formation markedly was the high air temperature.

It is clear from analysis of the frequency of occurrence of *Phytophthora* species in the sampled plant material (Table 4) that the dominant species was *P. citricola* (c. 60%), followed by *P. citrophthora* (c. 5%). *P. ramorum* had been reported in previous years on pieris, rhododendron and heather (Orlikowski, Szkuta 2005, Orlikowski *et al.*, 2006).

Colonisation of plant species by *Phytophthora. ramorum*

The colonisation by *P. ramorum* of the tissues of different plant species cultivated in nurseries and/or growing naturally had already been studied. The pathogen in question was found to be capable of causing disease in the majority of the rhododendron varieties, as well as in heathers, heaths and viburnum (Orlikowski *et al.* 2006). The data show how *P. ramorum* may spread from the said plants to other species growing at nurseries or in forests. It was also possible to demonstrate small differences in the level of pathogenicity of isolates from photinia, pieris, rhododendron and heather for these species of plants (Orlikowski *et al.* 2006).

The studies described here assessed colonisation of the tissues for heather, pieris, rhododendron, lilac, cowberry and viburnum by isolates of *P. ramorum* (Table 5) obtained from these plants in 2005. The detailed plant species are known from the literature as hosts of the pathogen. The research made use of the method from Orlikowski and Szkuta (2002).

Table 5. Colonisation of stems by *Phytophthora ramorum* 3 (a) and 5 (b) days after inoculation

Plant species	Source of isolates			
	Pieris japonica		*Rhododendron* sp.	
	a	b	a	b
Calluna vulgaris	10,6 c	18,8 b	11,5 c	20,8 d
Pieris japonica	7,5 b	16,0 b	10,0 bc	17,5 c
Rhododendron spp.	14,5 d	22,3 c	16,5 d	26,3 e
Sambucus nigra	5,5 a	8,8 a	6,5 a	10,3a
Vaccinium vitis-idaea	15,6 d	25,8 cd	18,8 e	28,5 e
Viburnum opulus Compactum	11,3 c	18,8 b	8,8 b	14,3 b

Note: Means in the columns denoted by the same letter do not differ significantly at the 5% level according to the Duncan test.

The isolate from pieris colonised the stems of 6 species of plants, albeit with necrosis developing most rapidly on the tissues of cowberry, most slowly on lilac (Table 5). The isolate from rhododendron also colonised the tissues of the plants studied, with necrosis developing fastest on cowberry and rhododendron (Table 5).

CONCLUSIONS

High air temperatures and a limited amount of rainfall left the 2006 growing season an unfavourable one for the development of *Phytophthora ramorum* on potential host plants at their natural sites, and also on allotments and in garden centres.

The infected shoots of pieris mainly yielded isolates of *P. citrophthora*, while those of rhododendron supplied *P. citricola*. The latter species was often associated with *Alternaria alternata, Botrytis cinerea, Fusarium avenaceum* and *Pestalotia sydowiana*. It is possible that the rapid growth of these species on media ensured that some of the colonies of *P. ramorum* originating from the inoculum became overwhelmed.

P. citricola and *P. citrophthora* were mainly isolated from rhododendron and pieris showing symptoms of stem-apical necrosis, as collected from 3 nurseries, as well as in part from plants cultivated on allotments or being offered for sale at garden centres. *Phytophthora* spp. were not reported from screened cowberry, heathers, beech or red oaks in forests.

The negative results for the attempted isolations of *P. ramorum* from infected parts of plants may reflect atmospheric conditions unfavourable for the development of the pathogen, and perhaps to an even greater extent its systematic elimination from nurseries.

Isolates of *P. ramorum* obtained in the previous year from infected apical shoots of pieris and rhododendron colonised the tissues of 6 plant species, albeit with necrosis developing fastest on cowberry and on rhododendron.

Literature

Aljanabi S. M. 1997: Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acid Res., 25: 4692-4693.

Boersma J. G., Cooke D. E. L., Sivasithamparam K. 2000: A survey of wildflower farms in the south-west of Western Australia for *Phytophthora* spp. associated with root rots. Australian J. Exper. Agricult., 40: 1011-1019.

Érsek T., Schoelz J. E., English J. T. 1994: PCR amplification of species-specific DNA sequences can distinguish among *Phytophthora* species. Appl. Environ. Microbiol., 60: 2616-2621.

Lee J. S., Lee P. O., Roh M. S. 1996: Classification of lilies using Random Amplified Polymorphic DNA (RAPD) analysis. Acta Horticulturae, 414: 137-143.

Lisek A. 2002: Opracowanie metod przechowywania pędów odmian truskawki i maliny w kulturach in vitro dla celów produkcji elitarnego materiału. Doctoral thesis, Research Institute of Pomology and Floriculture.

Ioos R., Husson C., Andrieux A., Frey P. 2005: SCAR-based PCR primers to detect the hybrid pathogen *Phytophthora alni* and its subspecies causing alder disease in Europe”. Eu. J. Plant Pathol., 112: 323-335.

Orlikowski L. B., Skrzypczak C., Szkuta G., 2006: *Phytophthora ramorum* – new invading plant pathogen in Poland. Scripta Horti Botanici, XI: 51-57.

Orlikowski L. B., Sroczyński M., Szkuta G. 2003: First notice of *Phytophthora* tip blight of *Calluna vulgaris*. Phytopathol. Pol., 31: 67-71.

Orlikowski L. B., Szkuta G. 2002: First record of *Phytophthora ramorum* in Poland. Phytopathol. Pol. 25: 169-179.

Orlikowski L. B., Szkuta G. 2005: *Phytophthora ramorum* w Polsce, stan aktualny i zagrożenia. Zeszyty Probl. Post. Nauk Rol., 504: 493-497.

Schubert R., Bahnweg G., Nechwatal J., Jung T., Cooke D. E. L., Duncan J. M., Müller-Starck G., Langebartels H., Sandermann H. Jr., Oßwald W. 1999: "Detection and quantification of *Phytophthora* species which are associated with root-rot diseases in European deciduous forests by species-specific polymerase chain reaction". Eur. J. For. Path., 29: 169-188.

Wiejacha K., Szkuta G., Orlikowska T., 2002: Optimization of DNA isolation procedure as the first step in identification of *Phytophthora* spp.. Bull. PAS, Biol. Sci., 50: 165-171.

REPORTS OF *PHYTOPHTHORA HEDRAIANDRA* ON *VIBURNUM TINUS* AND *RHODODENDRON CATAWBIENSE* IN SPAIN

Lassaad Belbahri[1], Eduardo Moralejo[2], Gautier Calmin[1], François Lefort[1],
Jose A. Garcia[2], Enrique Descals[2]

[1]Laboratory of Applied Genetics, School of Engineering of Lullier,
University of Applied Sciences of Western Switzerland, Jussy, Switzerland
[2]Instituto Mediterráneo de Estudios Avanzados, IMEDEA (CSIC-UIB), Miquel Marquès 21,
07190, Esporles, Balearic Islands, Spain

Phytophthora hedraiandra de Cock & Man in't Veld is a new species described from isolates recovered from infected leaves of *Viburnum tinus* in the Netherlands. It has since been reported in Italy causing stem canker and root rot on potted *V. tinus*, and blights on leaves and stems of *Rhododendron* in the USA. Herein we report the occurrence of *P. hedraiandra* on *Viburnum tinus* and *Rhododendron catawbiense* in Spain.

During *Phytophthora ramorum* surveys carried out at garden centres in Majorca, Spain in 2002, several potted plants of *Viburnum tinus* showing branch die-back were inspected (Moralejo *et al.* 2006). The origin of the lesions was traced to basal stem cankers ca. 10 cm above ground. The outer bark was aseptically removed and small pieces of tissue from the lesion front were plated onto a PARP selective medium. Two isolates, P3842 (= CBS 117808) and P3942 (= CBS 117809), were obtained and initially identified morphologically as *Phytophthora cactorum*. However, DNA sequencing identified these isolates as *P. hedraiandra*. The morphology of both isolates was therefore re-examined and compared with *P. cactorum* isolate P1611 (= CBS 117810) (AY943299).

Colony (on carrot agar CA; Brasier 1967) was slightly stellate with appressed to limited aerial mycelium (Fig. 1); on corn meal agar (CMA) the colony displayed a radiate and submerged pattern; on malt extract agar (MEA) the pattern was uniform or faintly stolonate and velvet; and on potato dextrose agar (PDA) it was uniform to slightly petaloid with felted to appressed mycelium. Radial growth rates on CA and CMA at 20°C were 8.5 mm d-1 and 5.5 mm d-1, respectively. No chlamydospores or sporangia were formed on any media, but sporangia did appear when mycelial plugs on CA were submerged in soil extract for 3 d at 20°C. Gametangia formed profusely, within 4 days on CA even on the aerial mycelium. The morphology of sporangia and gametangia of both isolates (Fig. 2) corresponded to the species description by de Cock & Lévesque (2004). The isolates differed from *P. cactorum* mainly in the

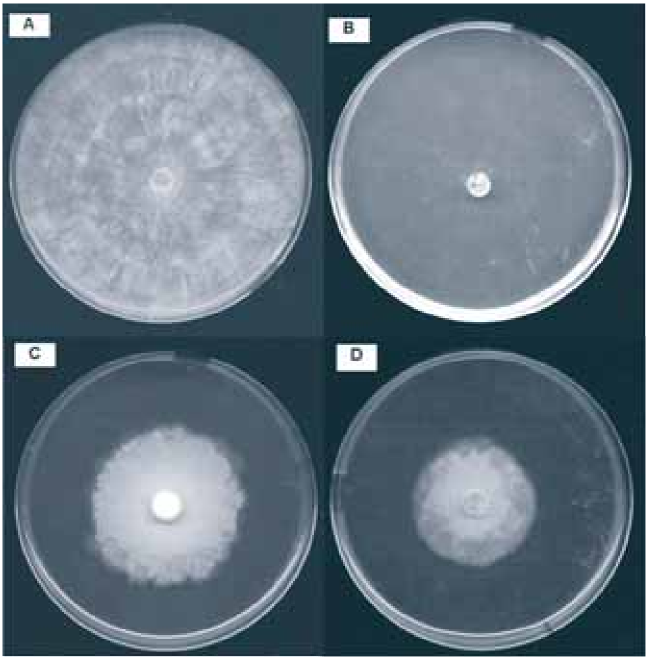


Figure 1. Colony pattern of *P. hedraiandra* (P3842) on CA (A), CMA (B), MEA (C) and PDA (D)

Figure 2. Morphology of *P. hedraiandra* (P3842) and *P. cactorum* (P1611). A) characteristic subglobose, papillate sporangia of *P. hedraiandra*; B) *P. cactorum* sporangial shapes; C) long stalked diclinous, paragynous antheridium of *P. hedraiandra*; and D) amphigynous antheridium of *P. cactorum*

Figure 3. Symptoms on *V. tinus* leaves seven days after wound-inoculating with *P. hedraiandra* (P3842); and (right) symptom on a twig of *V. tinus* 10 days after wound-inoculating with *P. hedraiandra* (P3942)

predominance of subglobose sporangia, near absence of tangled hyphae below the antheridia and larger oogonia and oospores.

Pathogenicity was determined by wound-inoculating the underside of detached leaves and twigs of *V. tinus* (wound made ca. 10 cm below the apex) with mycelial plugs. Controls were wound inoculated with sterile CA plugs. All leaves and twigs (Fig. 3) except the controls developed extensive necrotic lesions seven and 10 days after inoculating respectively, from which the fungus was re-isolated. This was the first finding of *P. hedraiandra* in Spain.

In June 2005, we observed a foliage lesion of a potted *Rhododendron catawbiense*. The symptom was a rounded, pale-brown water-soaked lesion on the margin of a young leaf. Isolation from the infected plant tissue was compared with an isolate used for morphological identification which conformed to *P. hedraiandra* (Fig. 4). Identity of this isolate was further confirmed by amplifying the mitochondrial Cox 1 gene and the

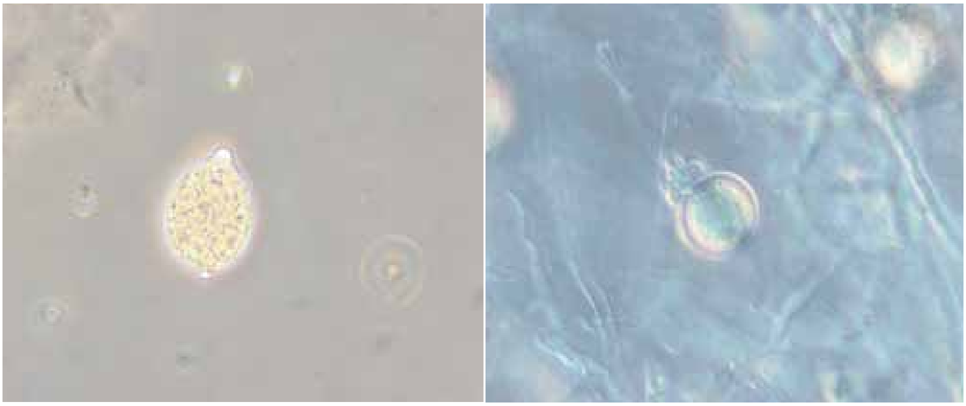


Figure 4. Sporangium (left) and oogonium and antheridium (right) of *P. hedraiandra* isolated from *Rhododendron catawbiense*

ITS region of the rDNA and subsequent sequencing, along with those of several new isolates recovered from leaves and stem lesions of *V. tinus* in December 2005.

The current emergence of *P. hedraiandra* has parallels with the early stages of the invasive alien species *P. ramorum*. It seems that this new pathogen is spreading worldwide within nurseries following the trade routes of *Viburnum* and *Rhododendron*. Its morphological similarities with *P. cactorum* could lead to an underestimation of its prevalence in nurseries where it could be misidentified. Further research is being carried out in order to determine its potential host range.

Reference

Brasier C. M. 1967. Physology of reproduction in Phytophthora. Hull, UK: University of Hull, PhD thesis.

De Cook A. W. A. M., Levesque A. 2004. New species of Pythium an Phytophthora. Studies in Mycology, 50: 481-488.

Moralejo E., Belbahri L., Calmin G., Lefort F., García J.A. and Descals E. 2006. First report of *Phytophthora hedraiandra* on *Viburnum tinus* in Spain. Plant Pathology, 55(4): 574.

THE INFLUENCE OF NURSERY-CULTIVATED PLANTS, AS WELL AS CEREALS, LEGUMES AND CRUCIFERS, ON SELECTED SPECIES OF *PHYTOPHTHOPRA*

Leszek B. Orlikowski[1], Tomasz Oszako[2]

[1]Institute of Pomology and Floriculture in Skierniewice; e-mail: lorlikow@insad.pl
[2] Forest Research Institute, Department of Forest Phytopathology, Sękocin,
05-090 Raszyn, Poland; e-mail: T.Oszako@ibles.waw.pl

INTRODUCTION

Intense cultivation of seedling in the forest nurseries degrades soil properties what is reflected in the gradual decrease of seedling health and its volnurability to pathogens attack (Łukaszewicz 2002). Crop rotation idea is derived from the principle that seedlings have different nutrition requirements. Species rotation change not only organic carbon content, but also lead to soil alkalization which favours development of *Phytophthora* spp. Such phenomenon might be especially important if there is a risk of introduction alien invasive species as *P. ramorum* (Orlikowski *et al.* 2006) or *P. karnoviae* (found some years ago in UK and quite recently in New Zeland). Because of quarantive rules and taking necessary precaution there is not possible to test influence of crop rotation on *P. ramorum* development directly, therefore for the experiment purpose another *Phytophthora* sp. had to be chosen. Taking into account the abundance of *P. citricola* in Polish nurseries (occuerring on such forest tree species as beech, fir, ash, oak and alder) this species seemed to be suitable and worth working with.

An application of the green fallow with different herbaceous species stimulate both the growth of seedlings and their phisiological conditions (Łukaszewicz and Duda 2000). Especially buckwheat, charlock and field pea have a positive influence on their size.There is hope that improvement of plant vitality may increase ressistance against *Phytophthora* infections. In Polish condition the highest risk of *P. ramorum* infection is for common beech, which species is considered to be volnurable to the attack of this pathogen and at the same time it is an important forest tree species being component of many forest stands.

The aim of the research carried out in the 2006 was to determine the influence of beech (*Fagus silvatica*), mustard (*Brasica juncea*), buckwheat (*Fagophyrum esculentum*), lupin (*Lupinus luteum*), sunflower (*Helianthus annuus*), vetch (*Vicia*

The research was supported by the State Committee for Scientific Research (KBN, Poland), grant 2P06L04128

sativa), rye (*Secale cereale*) and leaving fallow upon the dynamics of population *Phytophthora citricola* in soil, as well as the impact on the health of beech and alder (*Alnus glutinosa*) seedlings that remains of these plants exerted once ploughed in.

MATERIAL AND METHODS

Inoculum preparation

The selection of *P. citricola* for study reflected several years of work on the occurrence of *Phytophthora* species in nurseries. The species has often been isolated in nurseries, from beech, ash (*Fraxinus excelsior*), fir and alder. Work on the colonisation of plant tissues has also shown it to colonise leaves and stem parts of beech and alder very rapidly, albeit with the resultant necrosis developing much faster on alder (Table 1).

Table 1. The colonisation of leaves (a) and stems (b) of alder by *Phytophthora citricola,* isolate from beech

Plants	Days after inoculation			
	4		7	
	a	b	a	b
Fagus silvatica	8.5 a	15.3 a	14.8 a	24.5 a
Alnus glutinisa	22.3 b	24.0 b	36.8 b	38.5 b

Note: means in the columns denoted by the same latter do not differ significantly (at the 5% level), according to the Duncan test

The influence of nursery– cultivated plants on population dynamics of *Phytophthora citricola*

An experiment was founded on April 15th 2006, using 10-litre containers filled with a mixture of peat (1/3) and podsolic soil from the field (2/3). This earth had been infected with *P. citricola*, by applying the methodology given in Orlikowski (1999). The inoculum had been prepared in 90mm-diameter Petri dishes containing of oat medium (5 g of oat flakes + 18 ml of water). After 2 weeks, i.e. following the overgrowing of the medium by *P. citricola*, the inoculum was pulverised in a mixer, a small amount of distilled water having been added, before the pathogen suspension was mixed into small quantities of soil. This inoculum was then mixed in a cement mixer with the remainder of the soil. After 10 minutes of mixing, the earth was poured out into open plastic sacks, these then being subject to 10 days of incubation at c. 20°C. Prior to the filing of the containers, the soil was again subject to mixing in the cement-mixer. Containers were set out in the field, on black matting. Each container was then sown with seeds on May 4th 2006, the numbers ,varying in relation to the plant under study from 10 (in the case of sunflower and beech) to 50 (mustard). The control, and at the same time the ”fallow” containers comprised those not sown with seeds. The experimental layout entailed a random block design with 4 replications each of 5 containers.

The numbers of colony forming units (cfu) of *P. citricola* in the sown soil were determined, in relation to the plants used, after 0, 14, 44 and 75 days of cultivation, using selective medium based on gallic acid, as well as the methodology detailed in Orlikowski (1999). Soil taken from each container was ”sown” on to 4 Petri dishes with selective medium. The number of propagating units was calculated per gram air-dried mass of soil. The experiment was carried out using a completely randomised block design of 4 replications of 4 dishes each. The results were then processed statistically.

RESULTS

Fourteen days after the sowing of seeds, the abundance of *P. citricola* in soil was found to be varied, albeit with the fewest cfu being noted in those combinations in which the soil had been sown with the seeds of buckwheat, mustard, vetch and rye. By a significant margin, the most pathogen was to be found in the soil sown with germinating beech seeds (Table 2). The next 4 weeks brought an at least 11-fold increase in the abundance of *P. citricola,* probably in association with the positive influence on the pathogen’s development of shading of the soil, and perhaps also of the plants. Significantly the fewest propagating units of the pathogen were noted in soil following the cultivation of mustard, buckwheat and vetch. In turn, the most *P. citricola* – by a significant margin – was noted in the soil sown with beech and rye, as well as that without plants (Table 2).

After 75 days of the growth of plants in the different combinations, they were cut and broken up into very small parts. Samples of soil were then taken for analysis as regards *P. citricola* abundance, the remaining soil being mixed up with the plant fragments. Over the next two weeks, the containers were maintained in the field, being watered as necessary to ensure that drying-out of the soil did not take place.

Analysis of the abundance of propagating units in soil 75 days into the cultivation of plants (Table 2) again revealed differences in the populations of the pathogenic agent. Significantly the greatest numbers of units were present in the soil sown with beech, sunflower and vetch, the least in the combinations with mustard and buckwheat,

Table 2. The influence of rotation crops on changes in the size of the *Phytophthora citricola* population (Founded: 2006.05.04); number of colony growing units/g of air dry soil

Plants	Days after sowing		
	14	44	75
”Fallow”	88 bc	948 d	680 ab
Fagus silvatica	125 d	973 d	920 cd
Brasica juncea	50 a	589 a	620 a
Fagophyrum esculentum	50 a	666 ab	640 a
Lupinus luteus	94 c	794 bc	840 cd
Helianthus annus	81 bc	769 bc	960 d
Vicia sativa	75 a-c	718 ab	900 cd
Secale cereale	63 ab	871 cd	800 bc

Note: see Table 1

as well as the “fallow” soil. In these two combinations, the abundance of *P. citricola* was around a third lower than in soil in which beech, sunflower and vetch had been grown.

The influence of rotation crops on the germination and health of beech seedlings

This part of the research was in intimate linkage with the first concerning the influence of rotation crops on the dynamics to populations of *P. citricola*. The next stage of the research was proceeded with two weeks after the mixing of earth with pulverised plant parts or without sowing (”fallow”). Taken from the different combinations and pots was enough soil to fill 1-litre containers. These were placed out in the field on black matting under a net, before each pot was sown with five germinated beech seeds. The control comprised plants grown in non-infected soil, as well as in soil left “fallow”. Determinations of numbers of seedlings whose stems had appeared above ground were then made 28 days after sowing, before determinations of the numbers of seedlings displaying symptoms of *Phytophthora rot* were made after 28 and 46 days. The experiment was set out in a random block design with four replicates each featuring 5 germinated beech seeds.

To ensure whether the cause of the rot of stems from the apex down was *P. citricola* or something else, 5 mm fragments of the ailing stems were placed on potato-glucose (PDA). Over 24–96 hours of incubation, the dishes were looked at several times to determine whether the fragments of tissues had produced hyphae of *P. citricola*. The species was in fact found to be present in 92% of the tissue samples.

The data obtained show that 4 weeks following sowing was sufficient for between none and 1/3 of seedlings to show symptoms of *Phytophthorosa rot* (Table 3). Once a further 18 days had passed, there were no reports of ailing seedlings from the combination in which infected soil had been sown with buckwheat. In contrast, sporadic dieback of seedlings was being noted in the combinations with mustard and sunflower. Around half the seedlings died in the combination involving infected soil that had gone unsown (i.e. was “fallow”).

Analysis of the health of seedlings 75 days after sowing points to the data being very disparate (Table 3). All the emerged seedlings in the control combination (i.e. that in which beech was grown in non-infected soil) were healthy. The same (or at least a lack of visible disease symptoms) was true for half of the seedlings grown in soil with added buckwheat, as well as 2/5 of those growing up in the presence of lupin residues. In contrast, less than 1/10 of the seedlings survived in the combination in which the infected soil was not sown at all, as well as in that sown with beech seeds (Table 3).

The influence of rotation crops on the health of alder seedlings

The experiment used earth first sown and then mixed with different cultivated rotation crops, as in the studies concerning the health of beech seedlings. The experiment was laid out in a randomised block design with 4 replicates involving 20

Table 3. The influence of rotation crops on the germination and health of beech seedlings (Experiment founded 2006.08.01)

Plants	No. of germinated seedlings (n=5) 28 days after sowing	No. of dead seedlings at different numbers of days after sowing		No. of seedlings not displaying disease symptoms 70 days after sowing
		28	46	
Control no infection	4.8 a	0 a	0 a	4.8 e
”Fallow”	4.5 a	1.5 c	2.3 de	0.3 a
Beech seedlings	4.0 a	1.3 bc	1.8 cd	0.3 a
Brasica juncea	4.0 a	0.3 a	0.5 ab	1.0 ab
Fagophyrum esculentum	3.5 a	0 a	0 a	2.5 d
Lupinus luteus	3.5 a	0.5 ab	1.3 bc	2.0 cd
Helianthus annus	3.8 a	0 a	0.5 ab	1.3 bc
Vicia sativa	4.3 a	0.8 a-c	1.0 bc	1.0 ab
Secale cereale	4.0 a	0.8 a-c	2.8 e	0.5 ab

Note: see Table 1

seeds. The replicates took the form of four 1-litre containers, into each of which 5 seeds were sown. The containers were set out on black matting in the field, under a canopy, the numbers of emerging seedlings and their states of health being noted subsequently over a period of 70 days (Table 4).

Table 4. The influence of rotation crops on the health of alder seedlings grown for 70 days (Founded: 2006.08.04)

Plant	No. of emergent seedlings (n=20) after this no. of days		No. of seedlings (n=20) without symptoms of rot this no. of days after sowing	
	15	30	45	70
Control no infection	0.8 ab	12.0 bc	14.5 e	14.5 e
”Fallow”(infected control)	3.3 b	7.5 a	5.8 a	2.3 ab
Fagus silvatica	3.3 b	9.5 ab	6.3 ab	1.5 a
Brasica juncea	1.0 ab	11.8 bc	9.3 cd	7.8 d
Fagophyrum esculentum	1.0 ab	13.0 c	10.5 d	8.5 d
Lupinus luteus	0.5 a	12.3 bc	7.8 a-c	5.0 c
Helianthus annus	0.3 a	13.3 c	8.3 bc	4.5 c
Vicia sativa	1.3 ab	11.0 bc	6.5 ab	4.3 c
Secale cereale	6.5 c	11.0 bc	7.5 a-c	3.8 bc

Note: see Table 1

Observations made 15 days following the sowing of seeds revealed differences in seed germination. Most seeds had germinated where the sowing had taken place in contaminated soil mixed with parts of rye (Table 4). After 30 days, some 3/5 of seeds in the non-infected control combinations had germinated, as well as in the soil to which mustard, buckwheat, lupin, sunflower, vetch and rye residues had been added. The smallest numbers of plants had been obtained following the sowing of seeds into "fallow" soil (Table 4). Observations of the health of seedlings pointed to interdependence between the time of observation and the number of healthy alders. After 45 days, significantly the most healthy seedlings were to be noted in the non-infected control combination, and significantly the fewest in the "fallow" soil (Table 4). Around half of the seedlings were healthy where growth was in sown soil subsequently mixed with buckwheat and mustard.

Ten weeks after sowing, ailing seedlings were not reported at all in the control (non-infected) combination. Where *P. citricola* infection had been employed, the highest survival rates among seedlings characterised the combinations with residues of buckwheat and mustard, the lowest beech grown in the soil taken from the "fallow" conditions (Table 4).

CONCLUSIONS

1. Analysis of the influence of rotation crops on population dynamics of *Phytophthora citricola* in soil showed that the leaving fallow of soil, or else the cultivation therein of mustard and buckwheat, resulted in the most marked "lowering" of pathogen numbers in relation to those in the control. Data presented by Łabanowska (2006) make it clear that the tannins present in buckwheat, which probably pass out into the surrounding soil, are poisonous to beetle grubs. It seems probable that they also exert a negative impact on *P. citricola*. In turn, members of the *Cruciferae*, of which mustard is one, give rise to increased numbers of fluorescent bacteria of the genus *Pseudomonas*, which have a significant influence on the health of plants cultivated in rotation with them (Smolińska 2004). Likewise, the volatile compounds generated by the decomposition of glucosinolates, as liberated by decomposing mustard, may be toxic to fungi (Smolińska 2004), probably including *Phytophthora* spp.

2. The presence of buckwheat and lupins in soil infested by *P. citricola* brought about a significant obstruction of the development of beech seedling phytophthorosis. In combinations with these plants, there was a survival rate of more than 2/5 of seedlings. This compared with 1/5 or less in the remaining combinations.

3. Analysis of the influence of rotation crops on the health of alders pointed to buckwheat and mustard being most effective in curtailing the development of seedling *Phytophthora* rot. The combinations involving these plants allowed 2/5 of seedlings to survive, cf. 1/5 or fewer with other crops.

4. The data obtained would seem to speak for most frequent use of buckwheat in nursery rotation systems. This reflects, not only its impact in limiting the development of *P. citricola* (and most likely also other *Phytophthora* species), but also the way it disfavours the development of beetle grubs, which are among the more troublesome pests of nursery seedlings.

References

Łabanowska B. 2006: Pędraki and inne szkodniki glebowe in szkółkach. Szkółkarstwo 4: 96-100

Orlikowski L. B. 1999: Selective media for the evaluation of biocontrol agents efficacy in the control of soil-borne pathogens. Bull. Pol. Acad. Sci., Biol. Sci., 47: 167-172.

Orlikowski L. B., Skrzypczak C., Szkuta G., 2006: *Phytophthora ramorum* – new invading plant pathogen in Poland. Scripta Horti Botanici, XI: 51-57.

Smolińska U. 2004: Badania nad możliwością wykorzystania roślin *Brassicaceae*, zawierających związki biologicznie czynne, w ograniczaniu *Sclerotium cepivorum* Berk. Praca habilitacyjna, Monografie i rozprawy naukowe, Instytut Warzywnictwa, Skierniewice, 17: 1-79.

Łukaszewicz J. 2002. Zmiany zawartości składników pokarmowych w podłożu torfowo-korowym spowodowane zmianowaniem siewek podstawowych gatunków drzew leśnych. Prace Inst. Bad. Leś., A, 3 (937-943): 109-128.

Łukaszewicz J., Duda B. 2002. Uprawa sosny zwyczajnej w płodozmianach szkółki leśnej z zastosowaniem różnych ugorów zielonych. Prace Inst. Bad. Leś., A, 4(944-947): 21-42.

PHYTOPHTHORA POLONICA: A NEW SPECIES ISOLATED FROM A POLISH DECLINING ALDER FORESTS

Lassaad Belbahri[1], Gautier Calmin[1], Tomasz Oszako[2], Eduardo Moralejo[3], Jose A. Garcia[3], Enrique Descals[3], François Lefort[1]

[1]Laboratory of Applied Genetics, School of Engineering of Lullier,
University of Applied Sciences of Western Switzerland, Jussy, Switzerland
[2]Forest Research Institute (IBL), Department of Forest Phytopathology,
Sekocin Las, 05-090 Raszyn, Poland
[3]Instituto Mediterráneo de Estudios Avanzados, IMEDEA (CSIC-UIB),
Miquel Marquès 21, 07190, Esporles, Balearic Islands, Spain

In recent years, several Polish alder stands were found to be declining and an example of such stands is shown in figure 1. In a survey of *Phytophthora* associated with alder decline in Polish alder forests, several isolates of a homothallic *Phytophthora* sp., which could not be assigned to other taxa including *P. alni* subspecies, were consistently recovered from rhizosphere soil samples (Belbahri *et al.* 2006). Their morphology and pathogenicity, as well as sequence data for three nuclear

Figure 1. A dead alder forest in Koło district in Poland. This forest was planted 80 years ago and started very quickly dying 15 years ago

---

The research was supported by the State Committee for Scientific Research (KBN, Poland), grant 2P06L04128

regions (ITS rDNA, EF-1α and β-tub) and a coding mitochondrial DNA region (nadh1), were examined.

The new *Phytophthora* species forms catenulate, often lateral, hyphal swellings and large chlamydospores in agar media and in soil extract. Sporangia are ovoid to ellipsoid and non-papillate (Fig. 2). Large oogonia with paragynous and sometimes amphigynous antheridia were observed (Fig. 3). It is characterized by the moderate to

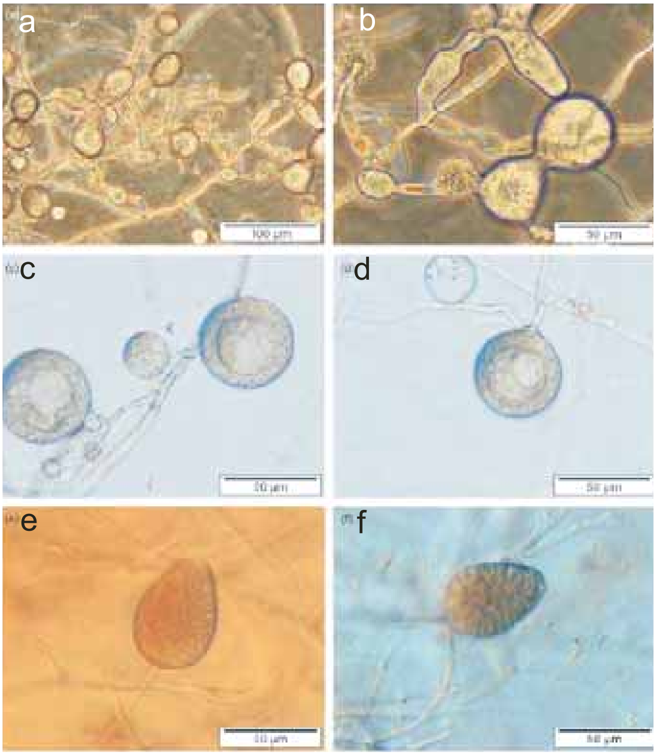


Figure 2. Asexual structures of *Phytophthora polonica* formed on CA. (a and b) Hyphal swellings (UASWS01 98). (c and d) Thin-walled chlamydospores on CMA (UASWS01 97). (e and f) Ovoid to ellipsoid sporangia (UASWS0197)

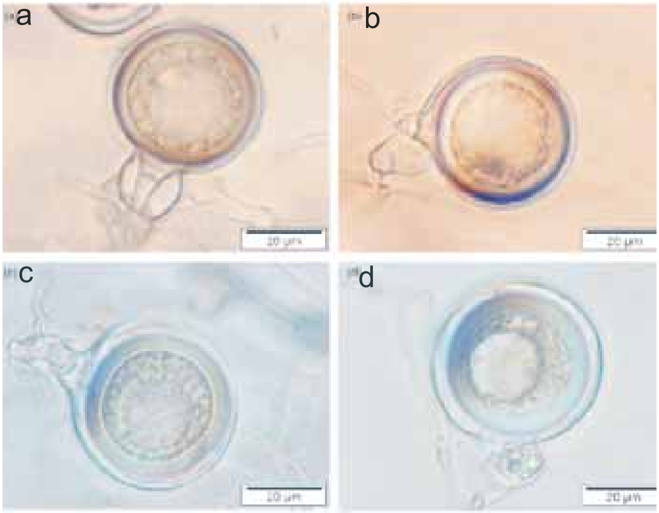


Figure 3. Gametangia of *Phytophthora polonica* UASWS01 98: (a) amphigynous antheridia; (b-d) spherical to subglobose oogonia with paragynous antheridia

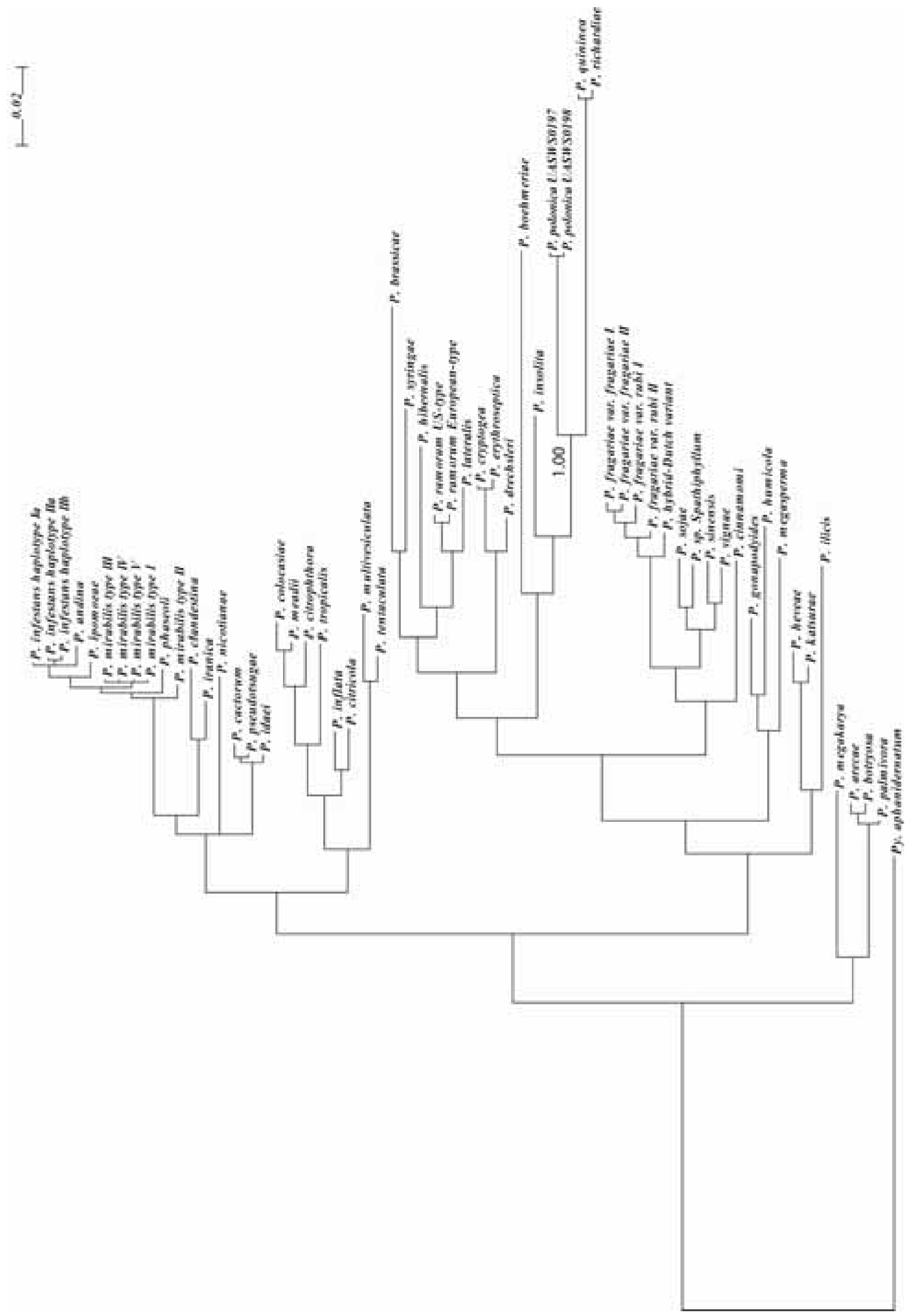


Figure 4. Bayesian inferences (BI) were obtained from concatenation of β-tub, EF-1α and nadh1 sequences with MrBayes v.3.1.2, using General Time Reversible (GTR) model allowing transitions and transversions to have potentially different rates. The program was run for 2 M generations, and sampled every 100 generations, with four simultaneous chains. The trees, sampled before the chains reached stationary, were discarded. Number at nodes show *a posteriori* probability

slow growth rate of its colony in carrot agar at 20°C, high optimal (ca. 30°C) and maximum (ca. 38°C) growth temperatures.

This new species was named *Phytophthora polonica* Belbahri L, Moralejo E & Lefort F. sp. nov.

The rDNA ITS sequences of eight *P. polonica* isolates had 100% identity and only 90% identity with their closest match *P. insolita* over an 824 bp sequence run. On the basis of the ITS sequence, *P. polonica* falls within 'clade 10' of Cooke *et al.* (2000), together with *P. insolita* and in clade 8 "sensu Kroon *et al.* (2004)" of *Phytophthora* (Fig. 4). *Phytophthora polonica* was slightly pathogenic to alder twigs and not pathogenic to trunks of several tree species. Above is presented a phylogenetic analysis using Bayesian inference (BI) for *P. polonica* and related species.

Reference

Belbahri L., Moralejo E., Calmin G., Oszako T., Garcia J. A., Descals E. and Lefort F. 2006. *Phytophthora polonica*, a new species isolated from declining alder stands in Poland. FEMS Microbiology Letters, 261:(2):165-174 .

Cooke D. E. L., Drenth A., Duncan J. M. Wagels G., Brasier C. M. 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. Fungial Genetics and Biology, 30: 17-32.

Kroon L. P. N. M., Bakker F. T., van den Bosch B. M., Bonants P. J. M., Flier W. G. 2004. Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. Fungial Genet. Biol., 41: 766-782.

POSSIBILITIES OF *PHYTOPHTHORA* IDENTIFICATION AS A GROUP OF NEW AND ECONOMICALLY IMPORTANT PATHOGENS IN FORESTRY

Oleg Baranov[1], Tomasz Oszako[2]

[1] Instytut Lasu Bialoruskiej Akademii Nauk, Homel
[2] Forest Research Institute, Department of Forest Phytopathology, Sękocin,
05-090 Raszyn, Poland; e-mail: T.Oszako.ibles.waw.pl

Phytophthora species are responsible for economically important diseases in a wide range of both agricultural and ornamental crops and forest species (Erwin, Ribeiro 1996). However, diagnosis and detection are obstructed by the fact that *Phytophthora* infection is frequently associated with stem-bases and roots. In addition, more than one species may be involved in a disease complex in some cases. Species identification in *Phytophthora* has traditionally been based upon microscopic examination of morphological characters and growth characteristics of the pathogen on specific media (Newhook *et al.* 1976). However, this type of (morphologically- and physiologically-based) identification of a pathogen is time-consuming and labour-intensive, and it requires specialised staff if it is to be performed correctly (Stamps *et al.* 1990). Furthermore, the obtainment of pure cultures from such substrates is challenging as *Phytophthora* species tend to be slow-growing on agar.

Notwithstanding such difficulties with identification (Shaw 1988), it has proved possible to distinguish more than 50 species formally, primarily on the basis of morphology. Once again, however, as variations in morphological characters of both the sexual and asexual stages of this group of pathogens exist, accurate identification by traditional methods poses problems (Waterhouse 1963).

Yet accurate and rapid identification of *Phytophthora* species in plant material is important for several reasons. First, many host plants are capable of becoming infected by multiple species of *Phytophthora*, with both the relative severity of the disease and the identity of the plant part infected varying from one pathogen species to another. Pre-plant identification may be important for quarantine purposes, and if the spread of pathogens in plant material is to be restricted. In addition, accurate diagnosis is obviously relevant to disease management and control (*Phytophthora* Threat Management, 2002).

The research was finansed by the National Fund of Environmental Protection an water Management on the demand of the Ministry of Evironment (grant no 198/06/Wn50/NE-PR-TxID)

Equally, a misidentification is to the detriment of both practical control and clear scientific communication. New species may have been wrongly assigned to current taxa, whilemorphological variants of existing taxa have doubtless been assessed incorrectly as new disease threats. Such problems, along with cases in which isolates are identified simply as "unknown *Phytophthora* sp." can delay appreciation that a new threat has arisen, resulting in a failure to offer suitable advice to growers (Guarro *et al.* 1999). By the same token, accurate identification is crucial to wider issues of plant health legislation and quarantine.

With a view to the efficiency and accuracy of the diagnostic methods used to distinguish different *Phytophthora* species being improved, work has been done to develop the dip-stick, agglutination, ELISA enzyme linked immunosorbent and IF assaying methods (Benson 1991). Where *Phytophthora* species are concerned, the most sensitive assay has proved to be the zoospore trapping immunoassay (ZTI). The dip-stick assay is only of use for *in vitro* testing, though dipsticks provided epidemiologically-valuable quantitative data on pathogen propagule numbers (Pettitt *et al.* 2002). Electrophoresis of the soluble proteins from mycelia is shown to be a useful aid in the identification and classification of various *Phytophthora* species (Bielenin *et al.* 1988; Kaosiri, Zentmyer 1986; Hansen, Brasier, Shaw, Hamm 1983; Erselius, de Vallavieille 1984). It has been proposed that several characteristics including protein profiles could supplement the strictly morphological characters being used as determinants in the identification and classification of *Phytophthora* species. The validity of protein banding patterns as a major determinant in the distinguishing of species and subgroups within a species has been highlighted by various workers (Bielenin *et al.* 1988; Kaosiri, Zentmyer 1986; Hansen *et al.*1983; Erselius, de Vallavieille 1984). Electrophoretic protein banding alone proved sufficient to allow the new species *P. pseudotsugae* to be described (Hamm, Hansen 1983).

Furthermore, in support of the electrophoretic techniques as a functional taxonomic criterion, protein profiles were employed as a major criterion in the discernment of P. *megasperma* subgroups (Hansen *et al.*1983). Electrophoretic protein profiles were found to be distinct for each species of *Phytophthora*, regardless of isolation date, geographical locality, age of the mycelium and growth medium employed (Bielenin *et al.* 1988; Kaosiri, Zentmyer 1986; Hansen, Brasier, Shaw, Hamm 1983; Erselius, de Vallavieille 1984; Hamm, Hansen 1983). Electrophoresis of native proteins was also shown to be useful in the separating and grouping of isolates from six species of *Phytophthora* encountered on deciduous fruit crops – which could also be distinguished by reference to cultural and morphological characters and cardinal temperature (Bielenin *et al.* 1988).

Isozyme analysis is also of value in studying the genetic variation that exists between and within fungal species. The expression of alleles coding for isozymes is generally not strongly dependent on the environment, nor is selection at such loci likely to be as intense and population-specific as that which occurs at loci for specific virulence. Isozyme analysis could also provide valuable genetic markers for use in future studies on *Phytophthora*, the lack of which has plagued geneticists specializing in the genus for many years now. Isozyme markers would be useful in allowing many individuals to be assayed on a single gel, since heterozygotes may be distinguished from homozygotes, and since isozyme banding patterns are not generally affected to any great extent by environmental factors. The assessment of genetic distances between isolates within species has assisted in *Phytophthora* systematics and studies regarding diversity (Oudemans and Coffey 1991a; Oudemans and Coffey 1991b).

Important technical advances have stimulated the use of molecular techniques of DNA analysis. First, the advent of DNA-markers has allowed for analysis of small numbers of cells or even single zoospores. Second, the selection of universal probes or primers specific to *Phytophthora* has provided easy access to nucleotide sequences.

Recently assuming a dominant role among these techniques are diagnostics based around the polymerase chain reaction (PCR). Tests have even been developed for some species of *Phytophthora* (Lee *et al.* 1993; Stammler, Seemuller 1993; White *et al.* 1990; Forster *et al.* 1995).

The recent development of genetic markers based on the PCR, such as random amplified polymorphic DNA (RAPD), has provided an unlimited number of markers for use in genetic studies. The DNA polymorphism in these markers arises from differences in sequences reflecting nucleotide-pair substitutions, deletions, inversions and translocations (Waugh and Powell 1992). While the same authors advocated the use of RAPD-derived fragments in the identification of *Phytophthora* species, the presence of such disadvantages to the method as dominant inheritance, a lack of prior knowledge on the identity of the amplification products and limited reproducibility have prevented wider adoption of the method. Another DNA fingerprinting technique — AFLP – might also prove useful where fine levels of genetic analysis in *Phytophthora* species are required (Ivors *et al.* 2004).

The amplified product can be cloned, sequenced and used to design specific longer primers, in order that a sequence characterized amplified region (SCAR) marker may be generated for use in identification of *Phytophthora* species (Martin *et al.* 2004). However, for many *Phytophthora* species, a lack of specificity to the primers proposed in literature is also reported. These primers can in fact give rise to cross-reactions with other species of *Phytophthora* and even with other very similar organisms, such as those of the *Pythium* or *Peronospora* groups (Tooley *et al.* 1997, Lacourt and Duncan 1997; Lindqvist *et al.* 1998; Loucourt *et al.* 1997, Schubert *et al.* 1999).

Single-strand-conformation polymorphism (SSCP) of ribosomal DNA in 29 species of *Phytophthora* was characterized in (Kong *et al.* 2004).

Molecular procedures for the PCR-RFLP based identification and detection of *Phytophthora* species have been developed and are in use in various laboratories in the US and Europe. Proposed methods include classical PCR based on the internal transcribed spacer (ITS) regions of ribosomal DNA (Hayden *et al.* 2004) and mitochondrial gene regions (Martin and Tooley 2004; Martin *et al.* 2004).

In conventional multiplex PCR, discrimination of amplified fragments relies on obtaining products of different sizes that can be distinguished by gel electrophoresis. However, different-sized products amplify with different efficiencies. By contrast, real-time specific detection methods allow for differentiation using fluorescent dyes, thereby allowing amplicons of the same length to be used. Real-time PCR chemistries utilized to detect and study phytopathogenic micro-organisms can be grouped into methods that are amplicon sequence-non-specific (SYBR Green) or sequence-specific

(TaqMan, Molecular Beacons, Scorpion PCR, etc.) (Mackay *et al.* 2002). SYBR green is a non-specific dye that fluoresces when intercalated into double-stranded DNA, whereas amplicon sequence-specific methods are based on the labelling of primers or probes with fluorogenic molecules that allow for detection of a specific amplified target fragment (Thelwell *et al.* 2000). In both approaches, amplicons can be measured at an early stage of the reaction, when the rate of amplification is still in its linear phase, allowing for the quantification of the initial amount of target DNA (quantitative analyses). In plant pathology, there are only a few examples of multiplex PCR applications, and usually not more than two different targets are detected simultaneously (Hughes *et al.* 2006; Ippolito *et al.* 2004; Winton *et al.* 2002; Tooley *et al.* 2006). This is partially due to the difficulties related to the development of a multiplex quantitative PCR assay, and to the frequent reduction in sensitivity achieved in multiplex PCR compared with separate reactions. Real-time PCR based on specific methods combines the sensitivity of PCR with the specificity of nucleic acid hybridization, making Southern blots or sequencing to confirm the identity of the amplicons unnecessary. These recent techniques therefore eliminate the need for post-amplification processing steps, are faster and more easily automated and avoid the use of toxic ethidium bromide. Other significant advantages of real-time PCR are the reduced potential for cross-contamination of specimens and the higher sensitivity of the reactions (Mumford *et al.* 2000).

Successful primer design for detection of a pathogen requires that the target region be unique to the organism of interest and conserved across its populations. The internal transcribed spacer (ITS) region has been shown to be largely conserved within *Phytophthora* spp. but differs across species. Because the ITS sequence occurs in multiple copies in the genome, the target concentration is effectively increased, thereby increasing its value for diagnostic primers.

This lack of specificity was ascribed to the method employed to construct the PCR primers that are routinely derived from ITS regions of ribosomal RNA genes (Schubert *et al.* 1999). The ITS sequences in *Phytophthora* species may not be the best source for the development of species-specific PCR primers, because interspecific differences in this part of the sequence are too small (Cooke *et al.* 2000; Cooke and Duncan 1997).

The best molecular tool used in phylogenetic and species determination studies of Oomycetes is direct sequence analysis, which has included the investigation of large and small subunit ribosomal RNA genes (Van de Peer *et al.* 1996; Förster *et al.* 1990), mitochondrial DNA (Förster *et al.* 2000; Crawford *et al.* 1996) and internal transcribed spacer (ITS) regions of the rRNAgenes (Cooke and Duncan 1997; Förster *et al.* 2000; Crawford *et al.* 1996)

Recently, Cooke *et al.* (2000) reported an ITS-based molecular phylogeny that included 50 *Phytophthora* species and is thus the most comprehensive to date.

Literature

Benson D. M. 1991. Detection of *Phytophthora cinnamomi* in azalea with commercial serological assay kits. Plant Dis., 75:478–482.

Bielenin A., Jeffers S. N., Wilcox W. F. , Jones A. L. 1988. Separation by protein electrophoresis of six species of *Phytophthora* associated with deciduous fruit crops. Phytopathology, 78: 1402-1408.

Cooke D. E. L., Duncan J. M. 1997. Phylogenetic analysis of *Phytophthora* species based on the ITS1 and ITS2 sequences of ribosomal DNA. Myc. Res., 101: 667-677.

Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G., and Brasier, C. M. 2000. A molecular phylogeny of *Phytophthora* and related Oomycetes. Fungal Genet. Biol., 30: 17-32.

Crawford A. R., Bassam B. J., Drenth A., Maclean D. J., Irwin J. A. G., 1996. Evolutionary relationships among *Phytophthora* species deduced from rDNA sequence analysis. Mycol. Res. 100: 437-443.

Erselius L. J., de Vallavieille C. 1984. Variation in protein profiles of *Phytopthora*; comparison of six species; Trans. Br. Mycol. Soc., 83: 463-472.

Erwin D. C., Ribeiro O. K. 1996. *Phytophthora* Diseases Worldwide. APS Press, St. Paul, Minnesota, USA.

Förster H, Cummings M. P., Coffey M. D., 2000. Phylogenetic relationships of *Phytophthora* species based on ribosomal ITS 1 DNA sequence analysis with an emphasis on Waterhouse groups V and VI. Mycol. Res., 104: 1055-1061.

Förster H., Coffey M. D., Elwood H., Sogin M. L. 1990a. Sequence analysis of the small subunit ribosomal RNAs of three zoosporic fungi and implications for fungal evolution. Mycologia, 82: 306-312.

Forster H., Learn G., Coffey M. D. 1995. Towards a better understanding of the evolutionary history of the species of the genus *Phytophthora* using isozymes, DNA RFLP's, and rDNA spacer sequences. [In:] *Phytophthora* 150 (eds. L. J. Dowley, E. Bannon, L. R. Cooke, T. Keane, E. O'Sullivan). European Association for Potato Research. Boole, Ltd., Dublin, Ireland, 42–54.

Guarro J., Gene J. Stchigel A. M. 1999. Developments in fungal taxonomy. Clinical microbiology reviews, 454–500.

Hamm Ń. Â., Hansen Ĺ. Ě. 1983. *Phytophthora pseudotsugae*, a new species causing root rot of Douglas-fir; Can. J. Bot., 61: 2626-2631.

Hansen Ĺ. Ě., Brasier C. Ě., Shaw D. S., Hamm Ń. Â. 1983. The taxonomic structure of *Phytophthora* megasperma: Evidence for emerging biological species groups; Trans. Br. Mycol. Soc., 87: 557-573.

Hayden K. J., Rizzo D., Tse J., Garbelotto M. 2004. Detection and quantification of *Phytophthora ramorum* from California forests using a real-time polymerase chain reaction assay. Phytopathology, 94: 1075–1083.

Hughes K. J. D., Tomlinson J. A., Griffin R. L., Boonham N., Inman A. J., Lane C. R. 2006. Development of a one-step real-time PCR assay for diagnosis of *Phytophthora ramorum*. Phytopathology, in press.

Ippolito A., Schena L., Soleti Ligorio V., Yaseen T., Nigro F. 2004. Real time detection of *Phytophthora nicotianae* and *P. citrophthora* in citrus roots and soils. Eur. J. Plant Pathol., 110: 833–843.

Ivors K. L., Hayden K. J., Bonants P. J., Rizzo D. M., Garbelotto M. 2004. AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum*. Mycol. Res., 108: 378-92.

Kaosiri Ô., Zentmyer G. A. 1986. Protein, esterase, and peroxidase patterns in the *Phytophthora palmivora* complex from cacao. Mycologia, 72: 988-1000.

Kong P., Hong C. X., Tooley P. W., Ivors K., Garbelotto M., Richardson P. A. 2004. Rapid identification of *Phytophthora ramorum* using PCR-SSCP analysis of ribosomal DNA ITS-1. Letters in Applied Microbiology, 38: 433–439.

Lacourt I., Duncan J. M. 1997. Specific detection of *Phytophthora nicotianae* using the polymerase chain reaction and primers based on the DNA sequence of its eliciting gene ParA1. European Journal of Plant Pathology, 103: 73–83.

Lee S. B., White T. J., Taylor J. W. 1993. Detection of *Phytophthora* species by oligonucleotide hybridization to amplified ribosomal DNA spacers. Phytopathology, 83: 177–181.

Lindqvist H., Koponen H., Valkonen J. P. T., 1998. Peronospora sparsa on cultivated *Rubus arcticus* and its detection by PCR based on ITS sequences. Plant Disease, 82: 1304-1311.

Loucourt I., Bonants P. J. M., Van Gent-Pelzer M. P., Cooke D. E. L., Hagenaar-De Weerdt M., Surplus L., Duncan J. M. 1997. The use of nested primers in the polymerase chain reaction for the detection of *Phytophthora fragariae* and *Phytophthora cactorum* in strawberry. [In:] Proceedings of the 3rd international Strawberry Symposium, Veldhoven 1996, 165-167.

Mackay I. M., Arden K. E., Nitsche A. 2002. Real-time PCR in virology. Nucleic Acids Res., 30: 1292–1305.

Martin F. N., Tooley P. W. 2004. Identification of *Phytophthora* isolates to species level using restriction fragment length polymorphism analysis of a polymerase chain reaction-amplified region of mitochondrial DNA. Phytopathology, 94: 983–991.

Martin F. N., Tooley P. W., Blomquist C. 2004. Molecular detection of *Phytophthora ramorum*, the causal agent of sudden oak death in California, and two additional species commonly recovered from diseased plant material. Phytopathology 94: 621-631.

Mumford, R. A., Walsh, K., Barker, I., Boonham N. 2000. Detection of potato mop top virus and tobacco rattle virus using a multiplex realtime fluorescent reverse-transcription polymerase chain reaction assay. Phytopathology, 90: 448–453.

Newhook F. J., G. M. Waterhouse, Stamps O. J. 1976. Tabular key to the species of *Phytophthora*. Mycotaxon, 32:199–217.

Oudemans P., Coffey M. D. 1991a. Isozyme comparison within and among worldwide sources of three morphologically distinct species of *Phytophthora*. Mycol. Res., 95: 19-30.

Oudemans P., Coffey M. D. 1991b. A revised systematics of twelve papillate *Phytophthora* species based on isozyme analysis. Mycol. Res. 95: 1025-1046.

Pettitt T. R., Wakeham A. J., Wainwright M. F., White J. G. 2002. Comparison of serological, culture, and bait methods for detection of *Pythium* and *Phytophthora* zoospores in water. Plant Pathology, 51(6): 720-727.

Phytophthora Threat Management. Department for Environment and Heritage 2002 (Standard Operating Procedure), 1–48.

Schubert R., Bahnweng G., Nechwall G. J., Jung T., Cooke D. E. L., Duncan J. M., Müller-Stark G., Langebartels C., Sandermann H. Jr., Oßwald W. 1999. Detection and quantification of *Phytophthora* species, which are associated with root-rot disease in European deciduous forests by species-specific Polymerase Chain Reaction. European Journal of Forest Pathology, 29: 169-188.

Shaw D. S. 1988. The *Phytophthora* species. Adv. Plant Pathol., 6: 27-51.

Stammler G., Seemuller E. 1993. Specific and sensitive detection of *Phytophthora fragariae* var. *rubi* in raspberry roots by PCR amplification. J. Plant Dis. Prot., 100: 394–400.

Stamps D. J., Waterhouse G. M., Newhook F. J., Hall G. S., 1990. Revised Tabular key to the species of *Phytophthora*. 2nd Ed. Mycological Paper No. 162, Commonwealth Mycological Institute, Kew, UK.

Thelwell N., Millington S., Solinas A., Booth J., Brown T. 2000. Mode of action and application of Scorpion primers to mutation detection. Nucleic Acids Res., 28: 3752–3761.

Tooley P. W., Bunyard B. A., Carras M. M., Hatziloukas E. 1997. Development of PCR primers from internal transcribed spacer region 2 for detection of *Phytophthora* species infecting potatoes. Appl. Environ. Microbiol., 63:1467-1475.

Tooley P. W., Martin F. N., Carras M. M., Frederick R. D. 2006. Real-time fluorescent polymerase chain reaction detection of *Phytophthora ramorum* and *Phytophthora pseudosyringae* using mitochondrial gene regions. Phytopathology, 96: 336–345.

Van de Peer Y., van der Auwera G., De Wachter R., 1996. The evolution of stramenopiles and alveolates as derived by "substitution rate calibration" of small ribosomal subunit RNA. J Mol. Evol., 42: 201-210.

Waterhouse G. M. 1963. Key to the species of *Phytophthora* de Bary. Commonwealth Mycological Institute, Kew, U.K. Mycological Paper, No. 92.

Waugh R., Powell W. 1992. Using RAPD markers for crop improvement. Trends in Biotechnology. 10/6: 186-191.

White T. J., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. [In:] PCR protocols: a guide to methods and applications (eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White). Academic Press, Inc., New York, N.Y, 315–322.

Winton L. M., Stone J. K., Watrud L. S., Hansen E. M. 2002. Simultaneous one-tube quantification of host and pathogen DNA with real-time polymerase chain reaction. Phytopathology, 92: 112–116.

NEW *PYTHIUM* SPECIES: *PYTHIUM QUERCUM, PYTHIUM STERILUM, PYTHIUM SPICULUM*

Lassaad Belbahri[1], Gautier Calmin[1], Tomasz Oszako[2],
Eduardo Moralejo[3], Esperanza Sanchez-Hernandez[4], Adele McLeod[5],
Enrique Descals[3], François Lefort[1]

[1]Laboratory of Applied Genetics, School of Engineering of Lullier,
University of Applied Sciences of Western Switzerland, Jussy, Switzerland
[2]Forest Research Institute, Department of Forest Phytopathology, Sekocin Las, Raszyn, Poland
[3]Instituto Mediterráneo de Estudios Avanzados, IMEDEA (CSIC-UIB), Miquel Marquès 21,
07190, Esporles, Balearic Islands, Spain
[4]Agroforestry Pathology, Department of Agronomy, University of Cordoba, Cordoba, Spain
[5]Department of Plant Pathology, University of Stellenbosch, Private Bag X1,
Matieland 7602 Stellenbosch, South Africa

We present here the morphology and molecular taxonomy based on the ITS region of the rDNA of three new species of *Pythium*: *Pythium quercum*, *Pythium sterilum* and *Pythium spiculum*, associated with forest decline.

Pythium sterilum Belbahri L & Lefort F. sp. nov. (Fig. 1 and Fig. 2)

In a survey of *Phytophthora* species associated with forest decline in Spain, Poland and France, we found three *Pythium* isolates, which have been characterized with internal transcribed spacer rRNA gene sequences and with classical morphological descriptors for *Pythium* spp. These isolates showed unique internal transcribed spacer sequences, different enough from those of any described species to justify new species status. These three distinct isolates failed to produce any sex organs with an entirely asexual reproduction and were found to represent a new species for which the name *Pythium sterilum* is proposed (Belbahri *et al.* 2005, 2006).

Pythium quercum Belbahri L. & Lefort F. sp. Nov (Fig. 3)

In a survey of the genus *Pythium* associated with forest decline in Spain, Poland, France and South Africa we found ten isolates of *Pythium*, which are very close to each other in morphological details and have a 99–100% similarity in the ITS sequence of the rDNA. The ITS region of these isolates is unique and different enough from those of

---

The research was supported by the State Committee for Scientific Research (KBN, Poland), grant 2P06L04128

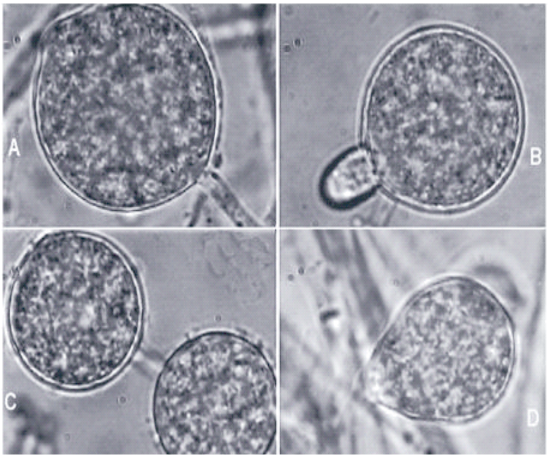


Figure 1. *Pythium sterilum*: (A) Terminal sporangia; (B) sporangia with an apical papilla; (C) intercalary sporangia and (D) terminal pyriform sporangia.

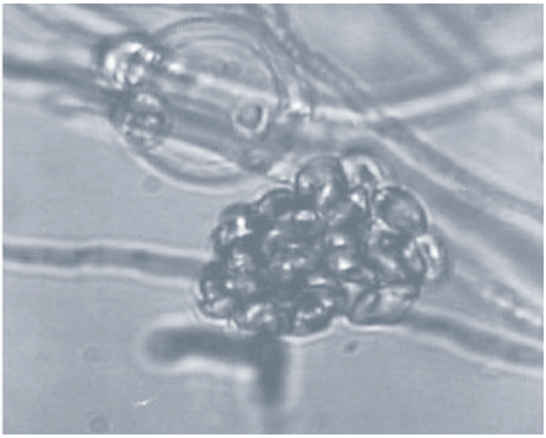


Figure 2: Formation and dehiscence of the sporangial vesicle in *Pythium sterilum*

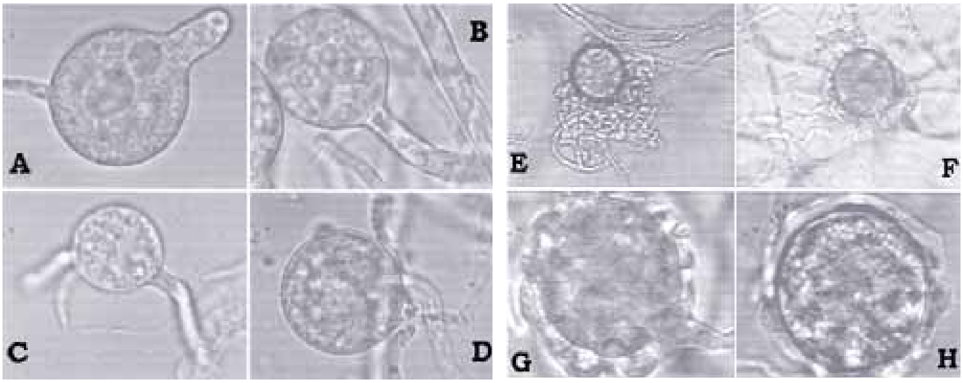


Figure 3. *Pythium quercum;* A–D: Sporangia germinating through germ tubes; E–H: Antheridia surrounding oogonia

Figure 4. Asexual and sexual reproductive bodies of *Pythium spiculum*: (A) peanut-shaped intercalary hyphal bodies; (B, D) intercalary elongated hyphal bodies; (C) spherical hyphal body

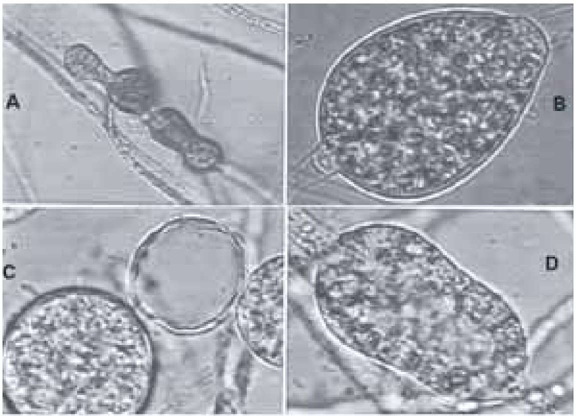
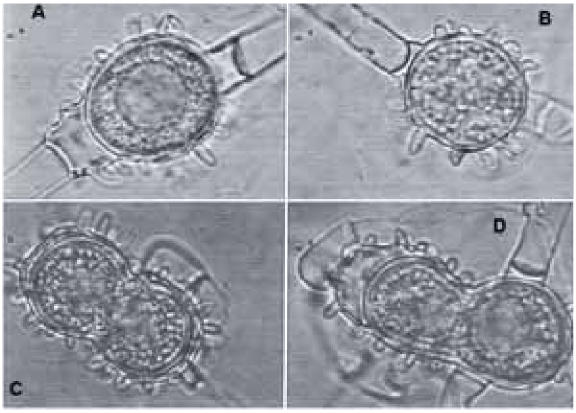


Figure 5. Spherical and intercalary ornamented oogonia (A, B); elongated to peanut-shaped oogonia with dumbbell -shaped oospores and monoclinous stalked antheridia (C, D)



any described species to justify the creation of a new taxon. The ten isolates, coming from 4 different geographic locations, reproduce asexually through sporangia. Sexual organs are rarely formed and when formed, the oospores do not mature.

***Pythium spiculum* (Fig. 4 and Fig. 5)**

Pythium spiculum was isolated from soil samples taken in different locations in France, Portugal and Spain (Paul *et al.* 2006). It has spiny oogonia and does not sporulate readily. It resembles *P. mamillatum* Meurs, but has its own distinguishing characteristics. It also exhibits sickle-shaped as well as spherical appressoria which at times are associated with sex organs like those found in *P. abappressorium* Paulitz and *P. contiguanum* Paul. Morphological description and DNA sequencing of the ITS region of its nuclear rDNA indicated a new species status for this organism, which looks to be related to *Pythium* species forming ornamented or spiny oogonia like *P. mamillatum*, *P. spinosum* and *P. irregulare* but also with those producing smoothwalled oogonia like *P. paroecandrum*, *P. sylvaticum* and *P. cylindrosporum* (Fig. 6).

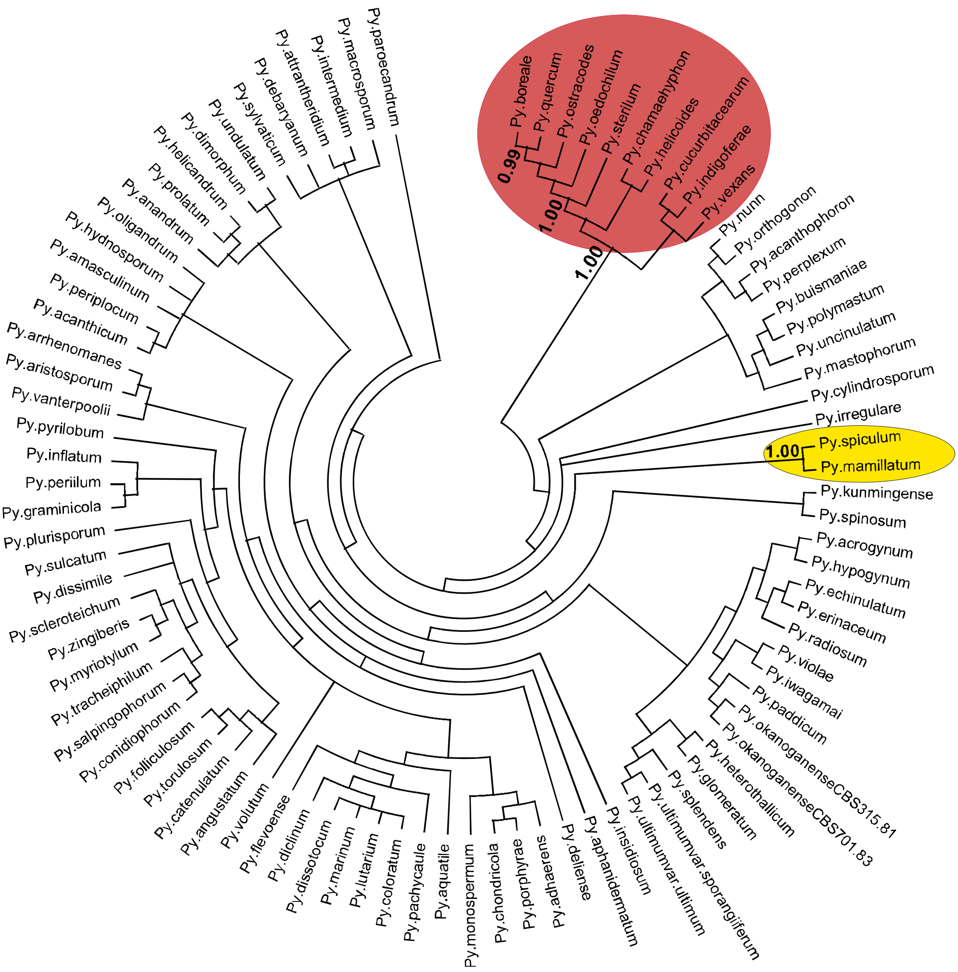


Figure 6. Unrooted circle showing Bayesian Inferences (BI) obtained from internal transcribed spacer sequences processed with MrBayes v.3.1.2. Number at nodes show posterior probability.
In red is shown the phylogenetic position of the *Pythium* clade K including *Pythium quercum* and *Pythium sterilum*. In yellow is given the phylogenetic position of the *Pythium* clade F including *Pythium spiculum*

References

Belbahri L., Oszako T., Calmin G., Sanchez-Hernandez E., Paul B., Lefort F. 2005. Characterization of *Pythium* spp. isolated from forest soils in Spain, Portugal and Poland and their possible implication in oak decline. [In:] Phytophthora in nurseries and forest stands (ed. T. Oszako). IBL Forest Research Institute, Warsaw, Poland. ISBN 83-87647-45-4.

Paul B., Bala K., Belbahri L., Calmin G., Sanchez-Hernandez E., Lefort F. 2006. A new species of *Pythium* with ornamented oogonia: morphology, taxonomy, internal transcribed spacer region of its ribosomal RNA, and its comparison with related species. FEMS Microbiology Letters, 254(2): 317-323.

Belbahri L., Calmin G., Sanchez-Hernandez E.,Oszako T., Lefort F. 2006. *Pythium sterilum* sp. nov. isolated from Poland, Spain and France: its morphology and molecular phylogenetic position. FEMS Microbiology Letters, Volume 255, Issue 2, February 2006, page 209-214.

FIRST RECORD OF *DISCULA DESTRUCTIVA* IN SWITZERLAND AND PRELIMINARY INOCULATION EXPERIMENTS ON NATIVE EUROPEAN *CORNUS* SPECIES

Ottmar Holdenrieder, Thomas N. Sieber

ETH Zurich, Forest Pathology & Dendrology, Institute of Integrative Biology (IBZ),
Universitätsstr. 16, CH-8092 Zürich, Switzerland; e-mail: ottmar.holdenrieder@env.ethz.ch

INTRODUCTION

Discula destructiva causes anthracnose and shoot dieback on dogwoods. It was first reported in 1978 in the states of New York and Connecticut (USA) on *Cornus florida*. Similar symptoms had also been observed on *C. nuttallii* on the west coast in 1976. The disease spread rapidly and caused serious damage to the forest ecosystem. First reports in Europe date back to 1995 when the disease was observed in the United Kingdom on *C. florida* imported from the USA (EPPO 2006). In 2002 first symptoms appeared in Germany (Stinzing and Lang 2003). In 2003 the fungus was observed on *C. florida* and *C. nuttallii* in a nursery in Lombardia, Italy (Tantardini *et al.* 2004). In Switzerland, the disease was detected for the first time in May 2006 on a *C. florida* planted as ornamental in a cemetery of Zurich. Therefore, we searched for presence of the disease on *C. florida* in other parks and for symptoms on native *Cornus* spp. in Switzerland and performed some preliminary inoculation experiments on *C. sanguinea* and *C. mas*.

METHODS

Seven major parks and cemeteries in or close to Zurich (ETH Hönggerberg, Kantonsspital park, cemeteries Hönggerberg, Nordheim, Enzenbühl, Sihlfeld and the Botanical Garden of the University of Zurich; distances approx. 1–14 km), and the arboretum Aubonne in western Switzerland (in a distance of approx. 140 km from Zurich) were visited during May to August 2006. Additionally, native *C. sanguinea* specimens at the edge of the forests Käferberg and Hönggerberg (close to the cemeteries Nordheim and Höngg) were repeatedly surveyed during the 2006 vegetation period. Symptomatic leaves and twigs from *Cornus* spp. were collected, incubated in moist chambers, and studied under the microscope. Isolations were made from conidia on MEA (20 g/L malt extract, 15 g/L agar, 50 mg/L terramycine), subcultures were incubated on MEA in diffuse daylight at room temperature. Pairings were made by placing inocula 1-3 cm distant from each other, and the reaction was evaluated after 4-5 weeks.

DNA was extracted according to Grünig *et al.* (2002). ITS regions were amplified with primers prITS4 and prITS5 (White *et al.* 1990) in a 30 mL reaction volume with ca. 1 ng template DNA. Single bands were gel purified with the QIAquik® gel extraction kit (QIAGEN, Basel, Switzerland), and sequences were determined with primer prITS4 at Microsynth (Balgach, Switzerland).

For the inoculation experiments, growing shoots (apical portion, approx. 10 cm long, without basal leaves) and detached fully developed leaves of *C. sanguinea* from different plants were used. Additionally, a few shoots of other potential host species were inoculated. The shoots were wounded 4–5 cm below the apex by removing approx. 2 mm^2 of the superficial bark tissues by a tangential cut with a scalpel. Small pieces of MEA (ca.1,5×1,5×1,5 mm) colonized by a sporulating colony of *D. destructiva* (strain 060516.2 from *C. florida*, Zürich) were used as inocula. After placing the inocula (upside down) on the host surface, a drop of sterile water was added to avoid desiccation. The samples were incubated in moist chambers at room temperature (approx. 22°C). Symptoms were noted and the infection was considered successful when sporulation of *D. destructiva* on necrotic lesions was detected.

RESULTS AND DISCUSSION

Discula destructiva was found in all locations where *C. florida* was present (seven of eight study sites, the only negative result was from the cemetery Enzenbühl were no *C. florida* was present) (Fig. 1, 2A). The morphology of our collections agrees very well with the descriptions of Redlin (1991) and Stinzing and Lang (2003). However, in our material the conidiophores were longer (up to 45 µm) and more intensely branched than shown by Redlin (1991). The ITS sequences of isolate 060516.2 (GenBank accession number EF088688) were identical with those of several strains of *D. destructiva* isolated from dogwood anthracnose in the USA (Zhang and Blackwell 2002).

Strains from different locations showed differences in cultural morphology (mycelial density, pigmentation, sporulation). No crystals [as described by Stinzing and Lang (2003)] were observed in our cultures. Conidia were formed in dark, more or less globular pycnidia-like structures (up to approx. 600 µm diameter) having 50–150 µm thick, partially indistinct walls composed of light brown textura intricata (hyphal diam. 2–5 µm), interspersed with single or locally aggregated blackish hyphae or hyphal cells.

The host plants were well established and had been growing at the sites for several years. The infected specimens showed leaf anthracnose and lower branch dieback as described by Stinzing and Lang (2003). The lesions started frequently on leaves on which fallen bracts became attached and created a moist environment. No infections were observed on the bracts themselves. Only one infected dead specimen of *C. florida* was observed, on the others symptoms remained largely restricted to lower branches. The relatively slow symptom development was probably caused by the warm and unusually dry weather in July 2006 (Schlegel and Albisser 2006), which also stopped a developing epidemic of *Apiognomonia veneta* on plane trees (Holdenrieder,

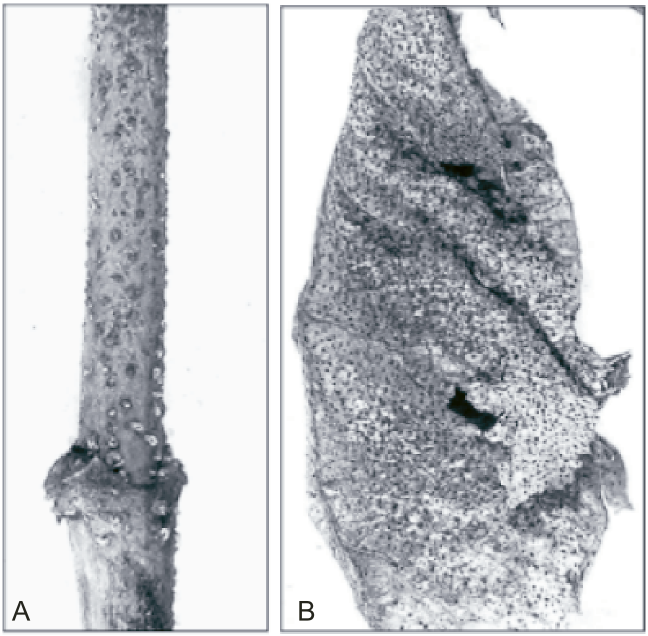


Fig. 1. Twig (A) and leaf (B) of *Cornus florida* covered with acervuli of *Discula destructiva*. The twig was incubated in a moist chamber and conidial cirrhi are extruded. The pictures depict dogwood anthracnose on tissues originating from the *C. florida* shrub on which the disease had been diagnosed for the first time in Switzerland (collection 060516.2 from cemetery Nordheim, Zurich)

unpublished). Moreover, most planted *C. florida* are in full light and therefore less predisposed to development of dogwood anthracnose [see Stinzing and Lang (2003)]. In the botanical garden, *C. nuttallii* and the hybrid *C. nuttallii x florida* were also affected. The rather frequently planted Japanese *C. kousa* exhibited anthracnose only in one case where it grew adjacent to a severely affected *C. florida*.

The pathogen sporulated abundantly in May on leaves from the previous year, which were still attached, as well as on dead twigs. Therefore, the disease must have been present already at least since 2005. In the arboretum Aubonne, dieback of *C. florida* has been observed for many years (S. Meier, pers. communication). *D.*

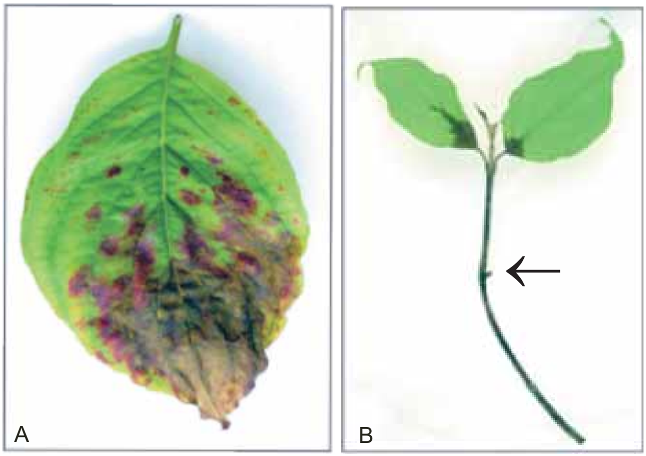


Fig. 2. Leaf symptom of *D. destructiva* infection on *Cornus florida* (A), and apical lesion formed after wound inoculation of a shoot of *C. sanguinea* (arrow: inoculum) (B)

destructiva can be transmitted by insects (Hed 1999) and probably also birds (Britton 1993). However, *C. florida* occurs in Switzerland at extremely low densities. Only single or very few specimens can be found in a given location and we believe that distribution by nursery stock seems more probable in this case. This view is supported by the results of our – very limited – vc-tests: Isolates from three different host trees within the cemetery – „Sihlfeld“ – were fully compatible whereas two pairings between strains from different locations showed demarcations. Genotypic diversity has also been observed in North America suggesting that multiple introductions may have occurred (Zhang and Blackwell 2002).

Genetically, *D. destructiva* forms a separate clade and seems to be an exotic species for North America as well as for Europe, sharing a recent ancestor with *D. umbrinella* (Caetano-Anolles *et al.* 2001). A teleomorph has never been observed, but might be located among Gnomoniaceae (Castlebury *et al.* 2002). From *C. sanguinea* in Switzerland, an *Apiognomonia rigniacensis* has been reported (Monod 1983), which should be included in further studies.

Host jumps after introduction of alien pathogens are a major threat for native plants (Slippers *et al.* 2005). Therefore, *Cornus* spp. in the vicinity of these locations were surveyed for the occurrence of the disease. Occasional shoot and branch dieback at very low severity was common on native *C. sanguinea.* In two instances, a girdling canker was observed on older stems. However, presence of *D. destructiva* could not be confirmed. Various other fungi sporulated on the dead branches (*Diplodia mamillana, Didymella corni, Seimatosporium* sp., *Cytospora* sp., *Pseudomassaria corni, Leioysphaerella vexata, Leptosphaeria* sp. and two unidentified coelomycetes). The bark cankers on older stems were colonized by *Cryptosporiopsis* sp. (GenBank accession number of the ITS sequences of strain 060514.1: EF088689) and *Didymella corni.* On a single specimen of *C. sericea*, which is widely planted and naturalized in Switzerland, pronounced shoot dieback was observed. The putative causal agent is a *Disculina*-like, probably undescribed coelomycete. *Cornus sericea* deserves particular attention, because it is rather suceptible to *D. destructiva* (Brown *et al.*1996). The only native *Cornus* species in Switzerland are *C. sanguinea* and *C. mas*; particularly *C. sanguinea* is very common and widespread. Exstirpation of this ecosystem component would have a major impact on biological diversity (Holzmueller *et al.* 2006).

Detached young wounded shoots and not fully developed leaves at the shoot apex of *C. sanguinea* were successfully inoculated with *D. destructiva* and the infections

Table 1. Results of inoculation experiments on *Cornus sanguinea* with *D. destructiva*

Experiment	Organ	Start	End	n	infected
1	shoot, wounded	3.6.06	23.6.06	5	3
2	shoot apex, not wounded	14.6.06	23.6.06	14	8
3a	adaxial leaf side, not wounded	14.6.06	12.7.06	8	0
3b	abaxial leaf side, not wounded	14.6.06	12.7.06	8	0

were associated with extensive necrotic lesions. Fully developed leaves were resistant (Table 1, Fig. 2B).

Additionally, on shoots of *C. mas* (n=4, from one individual) and *C. sanguinea* (n=6, from one individual), the cutting wound (cross section, approx. 10 cm distance from apex) was inoculated. The inoculation was successful on all shoots of *C. mas* and the fungus sporulated abundantly on this host, whereas in *C. sanguinea* only one shoot became infected (lesion length after 9 days: 27 mm). Two other shoots of *C. sanguinea* developed extensive stem lesions which were associated with *Pestalotiopsis* sp., three remained green (but *Botrytis cinerea* attacked the leaves). In addition, growing shoots of *Acer pseudoplatanus, Fagus sylvatica* and *Rubus fruticosus* aggr. (two of each species) were wound inoculated approx. 5 cm below the tip. Over the first two weeks, *D. destructiva* colonized only the wound surface of *Rubus* and no lesions were observed throught to the end of the experiment after 28 days.

In the field, *D. destructiva* was found only on *C. florida* and *C. kousa*. However, under very artificial conditions favouring the fungus, inoculation of *C. sanguinea* and *C. mas* was possible. From field observations in North America (Brown *et al.* 1996), *C. mas* is regarded as resistant; for *C. sanguinea*, no other data are available. Therefore, despite the apparent resistance of these species under field conditions, there may be some risk of a host jump. Therefore, careful monitoring and additional inoculation experiments are needed. Monitoring the health status of native *Cornus* spp. is costly, because these species are very common, sometimes difficult to identify and the occurrence of various other fungi complicates diagnosis. Also other Cornaceae (e.g. *Aucuba japonica, Davidia involucrata*) should be considered as potential hosts for *D. destructiva*.

Literature

Britton K. O. 1993. Anthracnose infection of dogwood seedlings exposed to natural inoculum in Western North-Carolina. Plant Dis., 77: 34-37.

Brown D. A., Windham M. T., Trigiano R. N. 1996. Resistance to dogwood anthracnose among *Cornus* species. J. Arboricult, 22: 83-86.

Caetano-Anolles G., Trigiano R. N., Windham M. T. 2001. Patterns of evolution in *Discula* fungi and the origin of dogwood anthracnose in North America, studied using arbitrarily amplified and ribosomal DNA. Curr. Genet., 39: 346-354.

Castlebury L. A., Rossman A. Y., Jaklitsch W. J., Vasilyeva L. N. 2002. A preliminary overview of the Diaporthales based on large subunit nuclear ribosomal DNA sequences. Mycologia, 94: 1017-1031.

EPPO, 2006. http://www.eppo.org/QUARANTINE/Alert_List/fungi/DISCDE.htm_(10.11.2006).

Grünig C. R., Sieber T. N., Rogers S. O., Holdenrieder O. 2002. Genetic variability among strains of *Phialocephala fortinii* and phylogenetic analysis of the genus *Phialocephala* based on rDNA ITS sequence comparisons. Can. J. Bot., 80: 1239-1249.

Hed B. E. 1999. Survival of conidia of *Discula destructiva* in frass of the convergent lady beetle. Plant Dis., 83: 806-809.

Holzmueller E., Jose S., Jenkins M., Camp A., Long A. 2006. Dogwood anthracnose in eastern hardwood forests: What is known and what can be done? J. Forestry, 104: 21-26.

Monod M. 1983. Monographie taxonomique des Gnomoniaceae (Ascomycetes de l'ordre des Diaporthales). Beihefte Sydowia, 9: 1-315.

Redlin S. C. 1991. *Discula destructiva* sp. nov. cause of dogwood anthracnose. Mycologia, 83: 633-642.

Schlegel T., Albisser P. 2006. Juli 2006. Klimatologisch ein extremer Monat. http://www.meteoschweiz.ch/web/de/wetter/wetterereignisse/juli_2006_erste_bilanz.html

Slippers B., Stenlid J., Wingfield M. J. 2005. Emerging pathogens: fungal host jumps following anthropogenic introduction. Trends Ecol. Evol., 20: 420-421.

Stinzing A., Lang K. J. 2003. Dogwood anthracnose. Erster Fund von *Discula destructiva* an Cornus florida in Deutschland. Nachrichtenbl. Deut. Pflanzenschutzd., 55: 1-5.

Tantardini A., Calvi M., Cavagna B., Zhang N., Geiser D. 2004. Primo rinvenimento in Italia di antracnosi causata da *Discula destructiva* su *Cornus florida* e *C. nuttallii*. Inform. Fitopatol., 12: 44-47.

White T. J., Bruns T. D., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. [In:] PCR Protocols: A Guide to Methods and Applications (eds M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White). Academic Press, Inc., Harcourt Brace Jovanovich, Publishers, New York, NY, pp 315-322.

Zhang N., Blackwell M. 2002. Population structure of dogwood anthracnose fungus. Phytopathology, 92: 1276-1283.

REDUCTION OF DIVERSITY IN ECTOMYCORRHIZAL FUNGAL FLORA CAUSED BY DAMAGE FROM PINE WILT DISEASE

Shin Ugawa[1], Kenji Fukuda

Institute of Environmental Studies, Graduate School of Frontier Sciences,
The University of Tokyo, Kashiwanoha 5-1-5, Kashiwa-shi, Chiba 277-8563, Japan
[1]Corresponding author (e-mail: shin-u@nenv.k.u-tokyo.ac.jp)

INTRODUCTION

Pine forests have been damaged heavily by pine wilt disease in eastern Asia. The cause of the disease is pine wood nematode *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle (Kiyohara and Tokushige 1971), which is known to be an invasive species from North America (Harmey and Harmey 1993, Tarès *et al.* 1993). The nematode is primarily vectored by a cerambycid beetle *Monochamus alternatus* Hope (Mamiya and Enda 1972, Morimoto and Iwasaki 1972). The beetle feeds on pine shoots in June and July and the nematode infests pine tree (e.g. Togashi 1988). The nematode causes cavitation in pine stem, and the infested pine trees wilt and die in July and August. The nematode is pathogenic not only to Asian pine species but also to European pine species (Bedker *et al.* 1987, Riga *et al.* 1991). Unfortunately, the nematode was detected in Portugal in 1999 (Mota *et al.* 1999).

In Japan, two pine species, *Pinus densiflora* Sieb. et Zucc. and *Pinus thunbergii* Parl. are highly susceptible to pine wilt disease. The damaged wood volume reached 2.43 million m^3/year at its peak in 1979, and Mamiya (1983) reported that 650,000 ha of pine forests, amounting to 25% of total pine forests in Japan, was damaged. Even now, the damaged volume reaches around 0.70 million m^3/year. Thus, the damage is so heavy and sudden to influence pine forest ecosystems, *e.g.* the diversity of other organisms.

Ectomycorrhizal fungi are a fungal group, which are symbiotic to tree species. Via ectomycorrhiza, which the fungi form with tree roots, soil minerals and water are transported to host tree, and inversely, the host tree gives carbohydrate to the fungi. Thus, ectomycorrhizal fungi, which are symbiotic to pine species, need to live with pine trees as the host. Therefore, the decline of pine forest caused by pine wilt disease may influence the diversity of ectomycorrhizal fungi.

The purpose of this study was to clarify the influence of pine wilt disease on the diversity of ectomycorrhizal fungi. Therefore, we surveyed the damage of pine stands caused by pine wilt disease and the occurrence of ectomycorrhizal fungal fruit bodies, and analyzed the relationship between them.

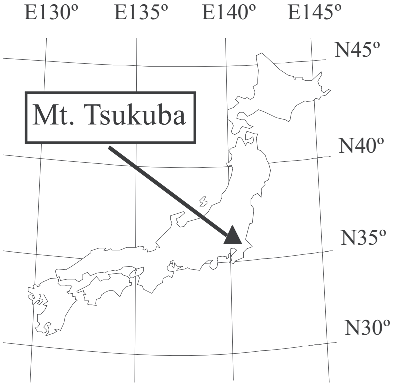


Fig. 1. Location of study site. Mt. Tsukuba (36°13'31" N, 140°06'24" E; altitude, 877 m) is 60 km northeast of Tokyo

STUDY SITE

The study site was situated in artificial pine stands in the neiberghood of Mt. Tsukuba (36°13'31" N, 140°06'24" E; altitude, 877 m) in Ibaraki prefecture, Japan (Fig. 1). The stands are composed of *P. densiflora* and *P. thunbergii* below altitude of 450 m.

METHODS

At the study site, eleven quadrats in pine stands with different level of damage were established in 1999 (Table). The size of each quadrat was 30 m by 20 m. In 1999 the quadrats were divided into two groups with different community structure. One group was almost pure pine, and named P1–9. Another group was mixed with *Quercus serrata* Thunb., and named M1–2. In 1999–2004 the quadrats in each group were re-numbered according to the average of annual mortality.

A tree census was conducted in all quadrats from September to February in 1999–2004. For each tree 3.0 m tall, we recorded the species, and measured the diameter at breast height. And, we checked whether pine individuals were live or dead according to discoloration of tree crown. We calculated the accumulative mortality of pine individuals and the total basal area (BA) of live pine trees.

The occurrence of fungal fruit bodies was surveyed in all quadrats from May to November in 1999–2004. Each quadrat was divided into 25×25-cm segments, and we recorded the species and the location of the segment with fruit bodies once per two weeks. In this study, seven fungal families, *Amanitaceae*, *Boletaceae*, *Cortinariaceae*, *Gomphidiaceae*, *Hygrophorus*, *Russulaceae* and *Strobilomycetaceae*, and one genus *Laccaria* were considered as ectomycorrhizal fungi. Other ectomycorrhizal genera (*e.g.* Tricholoma) were not observed.

STATISTICAL ANALYSIS

The number of live pine trees decreased drastically in July and August because pine wilt disease progresses under water stress in summer. So, from August in year to July in year t+1, pine population is considered to be in a steady state. Therefore, we compared the damage of pine trees with the number of species of ectomycorrhizal

Table. Community structure in each quadrat in 1999

Quadrat	P1	P2	P3	P4	P5	P6	P7	P8	P9	M1	M2
Size (m^2)	600	600	600	600	600	600	600	600	600	600	600
Altitude (m)	370	420	300	340	370	150	410	230	250	370	390
Aspect	SE	E	E	NW	W	NE	N	SE	N	NW	NE
Inclination (°)	14	17	21	19	18	5	23	26	11	25	12
Number of species	11	13	20	20	14	23	24	13	15	19	22
Number of stem per hectare	1800	1800	3217	2967	1850	2767	3400	1717	1933	3033	2650
BA per a hectare (m^2)	54,57	21,33	36,96	32,79	36,94	49,42	40,50	39,33	42,79	33,39	32,19
Max DBH (cm)	50,0	36,0	35,0	34,0	37,0	36,0	34,0	35,5	37,5	32,0	30,5
Dominant species[*1]	Pd/Co	Pd	Pd	Pd	Pd	Pd	Pd	Pd	Pd	Pd / Qs / Pt	Pd / Qs / Pj
Number of pine individuals[*2]	39	35	94	47	79	70	65	57	59	59	51
Accumulative mortality[*3] (%)	25,6	40,0	40,4	19,1	43,0	21,4	36,9	21,1	23,7	32,2	37,3
RBA of live trees											
Pinus densiflora	0,78	0,76	0,75	0,83	0,78	0,89	0,69	0,82	0,86	0,48	0,52
Pinus thunbergii	-	-	-	-	-	-	-	-	-	0,12	-
Mycorrhizal tree species[*4]	0,03	0,01	0,08	0,02	0,09	0,01	0,05	0,02	0,04	0,19	0,23
Other species	0,19	0,23	0,17	0,15	0,13	0,10	0,25	0,16	0,10	0,21	0,25
RBA of dead trees											
Pinus densiflora	0,99	1,00	1,00	0,97	0,99	0,99	0,93	0,86	1,00	0,98	0,94
Other species	0,01	0,00	0,00	0,03	0,01	0,01	0,07	0,14	0,00	0,02	0,06

[*1] Pd – *Pinus densiflora*, Qs – *Quercus cerrata*, Co – *Chamaecyparis obtusa*

[*2] For both live and dead trees in each quadrat

[*3] For only two pine species

[*4] Four genus: *Abies*, *Carpinus*, *Castanea*, *Quercus*

fungal fruit bodies, which occurred during July to November of the year and May to August of the next year.

RESULTS

In all quadrats, the accumulative mortality was 19.1–43.0% in 1999, and reached 33.3–94.3% in 2004 (Fig. 2). The average of annual mortality was 12.3% in the heaviest quadrat P1 and 2.4% in the lowest quadrat P9 (Fig. 3). In the quadrats with high annual mortality, *e.g.* quadrat P1 and P2, the BA of live pine trees decreased drastically (Fig. 4). However, in the quadrats with low annual mortality, *e.g.* quadrat P8 and P9, the BA sometimes increased.

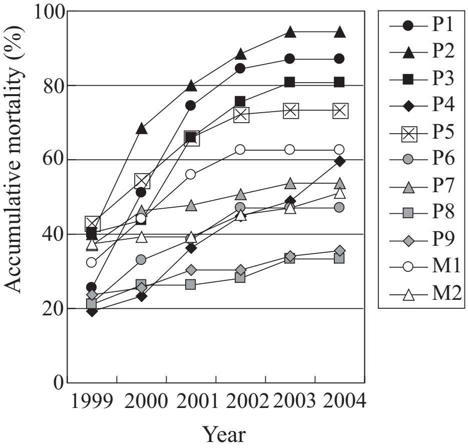


Fig. 2. Accumulative mortality of *Pinus* spp. in each quadrat in 1999–2004

The number of species of ectomycorrhizal fungal fruit bodies fluctuated among years (Fig. 5). In the quadrats with low annual mortality, *e.g.* P1 and P2, the number of species peaked in 2001. However, in the quadrats with high annual mortality, the number of species decreased every year.

The relationship between the accumulative mortality and the number of species of ectomycorrhizal fungal fruit bodies is shown in Figure 6. In the quadrats with low annual mortality, the number of species changed regardless of the accumulative mortality. However, in some quadrats with high annual mortality, *i.e.* P2, P3 and P5, the number of species significantly decreased with the accumulative mortality. This tendency was found among quadrats in 2002 and 2003 (Fig. 7).

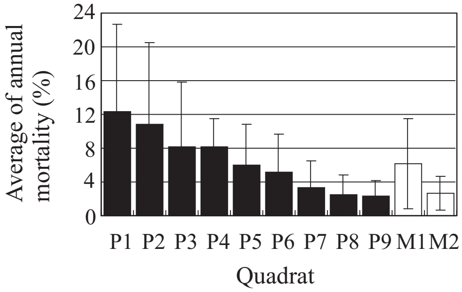


Fig. 3. The average of annual mortality of *Pinus* spp. in each quadrat

The number of species of ectomycorrhizal fungal fruit bodies significantly increased with the BA of live pine trees in the quadrats with high annual mortality, but not in the quadrats with low annual mortality (Fig. 8). Among quadrats, the correlation between the number of species and the BA was significant in 2002 and 2003 (Fig. 9). Moreover, in quadrat M1 and M2, the number of species was higher than that of other quadrats with the same BA. If the data of quadrat M1 and M2 were excluded, the correlation between the number of species and the BA was significant in 2001.

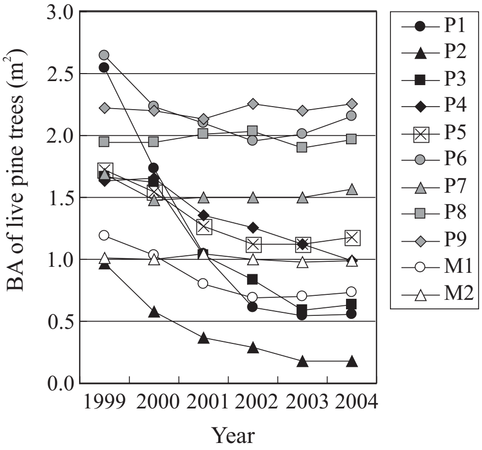


Fig. 4. Total basal area (BA) of live trees of *Pinus* spp. in each quadrat in 1999–200

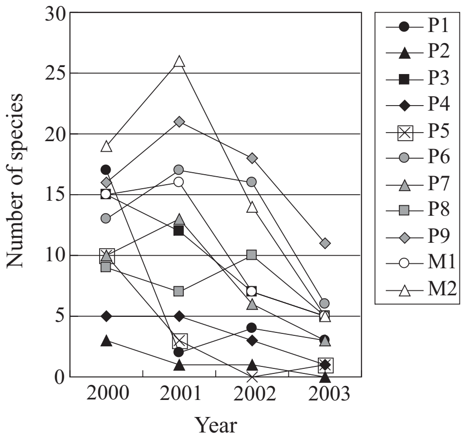
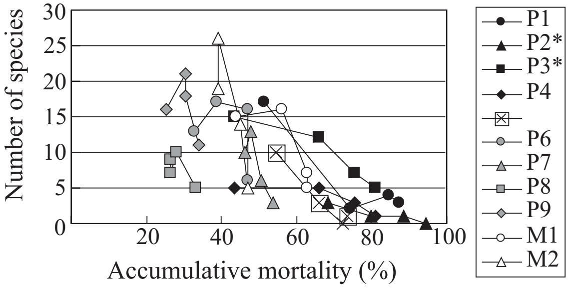


Fig. 5. The number of species of ectomycorrhizal fungal fruit body in each quadrat in 2000–2003

Fig. 6. The number of species of ectomycorrhizal fungal fruit body with accumulative mortality of *Pinus* spp. in each quadrat in 2000–2003



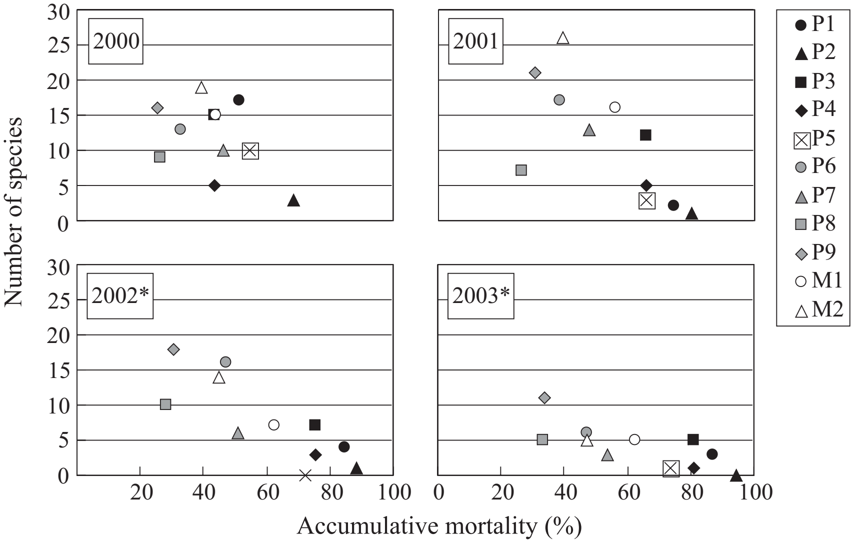


Fig. 7. The number of species of ectomycorrhizal fungal fruit body with accumulative mortality of *Pinus* spp. in each year

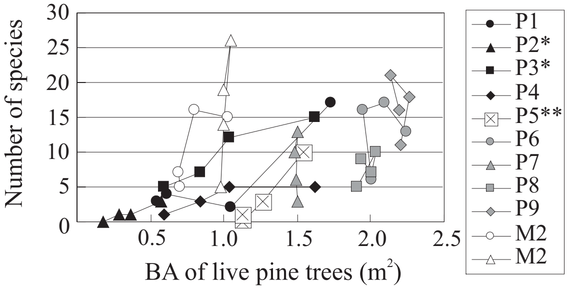


Fig. 8. The number of species of ectomycorrhizal fungal fruit body with total basal area (BA) of *Pinus* spp. in each quadrat in 2000–2003

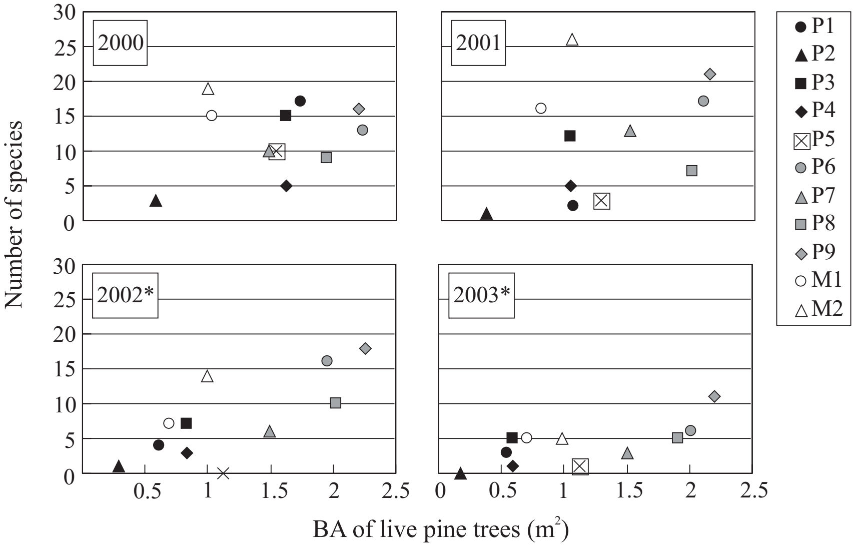


Fig. 9. The number of species of ectomycorrhizal fungal fruit body with total basal area (BA) *Pinus* spp. in each year

DISCUSSION

The biomass of live pine trees decreases in heavily damaged pine stands (Fig. 4). However, the biomass of live pine trees sometimes increases in slightly damaged pine stands. It is thought that the growth of pine individuals could compensate for the damage if the damage was not severe. Thus, the damage by pine wilt disease was divided into categories of heavy damage with a decrease of biomass and light damage no decrease or even an increase in biomass.

It is clear that the diversity of ectomycorrhizal fungi is maintained in the case of light damage (Fig. 6–9) and it is suggested that the constant amount of host pine trees retains the supply of carbohydrate for ectomycorrhizal fungi. Inversely, the diversity of ectomycorrhizal fungi reduces with heavy damage to pines(Fig. 6–9). Power (1996) found that the amount of ectomycorrhizas in declining trees was less than that in healthy ones. Ichihara *et al.* (2001) reported that the development of ectomycorrhizas stops after the inoculation into trees of *Bursaphelenchus xylophilus*, the pathogen of pine wilt disease. Therefore, the loss of host pine trees arising from heavy damage is thought to be causing the reduction of the diversity of ectomycorrhizal fungal flora in the study area.

The diversity of ectomycorrhizal fungi was kept high in pine stands mixed with other ectomycorrhizal tree species (Fig. 8–9). Some species of ectomycorrhizal fungi can associate with a wide range of tree species. Thus, the other ectomycorrhizal tree species can provide refugia for ectomycorrhizal fungi when pine trees are damaged.

Acknowledgements. *We are indebted to the members of the Department of Natural Environmental Studies, Graduate School of Frontier Sciences, The University of Tokyo for help with this research. We thank Dr. K. Suzuki (Graduate School of Bioresource Sciences, Nihon University) for supporting this research. Permission to study this National Forest site was provided by the Forestry Technology Center, Kanto Regional Forest Office, Forestry Agency. This research was supported by the JSPS Grants-in-Aid No. 18380088 for Scientific Research and the ESPEC Foundation for Global Environment Research and Technology (Charitable Trust).*

References

Bedker P. J., Wingfield M. J., Blanchette R. A. 1987. Pathogenicity of *Bursaphelenchus xylophilus* on three species of pine. Canadian Journal of Forest Research, 17: 51–57.

Harmey J. H. and Harmey M. A. 1993. Detection and identification of *Bursaphelenchus* species with DNA fingerprinting and polymerase chain reaction. Journal of Nematology, 25: 406–415.

Ichihara Y., Fukuda K., Suzuki K. 2001. Suppression of ectomycorrhizal development in young *Pinus thunbergii* trees inoculated with *Bursaphelenchus xylophilus*. Forest Pathology, 31: 141–147.

Kiyohara T., Tokushige Y. 1971. Inoculation experiments of a nematode, Bursaphelenchus sp. Onto pine trees. Journal of Japanese Forestry Society, 53: 210–218 (in Japanese with English summary).

Mamiya Y. 1983. Pathology of the pine wilt disease caused by *Bursaphelenchus xylophilus*. Annual Review of Phytopathology, 21: 201–220.

Mamiya Y., Enda N. 1972. Transmission of *Bursaphelenchus lignicolus* (Nematode: Aphelenchoididae) by *Monochamus alternatus* (Coleoptera: Cerambycidae). Nematologica, 18: 159–162.

Morimoto K., Iwasaki A. 1972. Role of *Monochamus alternatus* (Coleoptera: Cerambycidae) as a vector of *Bursaphelenchus lignicolus* (Nematode: Aphelenchoididae). Journal of Japanese Forestry Society, 54: 177–183 (in Japanese with English summary).

Mota M. M., Braasch H., Bravo M. A., Penas A. C., Burgermeister W., Metge K., Sousa E. 1999. First report of *Bursaphelenchus xylophilus* in Portugal and in Europe. Nematology, 1: 727–734.

Power S. A., Ashmore M. R. 1996. Nutrient relations and root mycorrhizal status of healthy and declining beech (*Fagus sylvatica* L.) in southern Britain. Water, Air and Soil Pollution, 86: 317–333.

Riga E., Sutherland J. R., Webster J. M. 1991. Pathogenicity of pinewood nematode isolates and hybrids to scots pine seedlings. Nematologica, 37: 285–292.

Tarčs S., Lemontey J.M., De Guiran G., Abad P. 1993. Cloning and characterization of a highly conserved satellite DNA sequence specific for the phytoparasitic nematode *Bursaphelenchus xylophilus*. Gene, 129: 269–273.

Togashi K. 1988. Population density of *Monochamus alternatus* adults (Coleoptera: Cerambycidae) and incidence of pine wilt disease caused by *Bursaphelenchus xylophilus* (Nematode: Aphelenchoididae). Researches on Population Ecology, 30:177–192.

A REVIEW OF HISTORICAL DATA ON SELECTED ALIEN INVASIVE PATHOGENS AND PESTS IN EUROPE

Magdalena Basiewicz[1], Dobrawa Jankiewicz[1], Steve Woodward[2],
Nikoleta Soulioti[2], Tomasz Oszako[1]

[1]Forest Research Institute Department of Forest Pathopathology,
Sękocin Stary, ul. Braci Leśnej 3, 05-090 Raszyn, Poland
[2]University of Aberdeen, School of Biological Sciences, Department of Plant and Soil Science,
Cruickshank Building, Aberdeen AB24 3UU, Scotland, UK

INTRODUCTION

Forest ecosystems and individual trees in Europe are threatened by a wide range of pests and diseases. Previous experience indicates that many of these organisms have the potential to cause serious damage, particularly when introduced into ’new’ environments. This review is focused on some of the major threats to trees in Europe, especially diseases, but also including some examples of recently invading invertebrates. We include a number of diseases (and pests) already present on this continent and several which may have serious consequences if/when they are introduced.

WILT DISEASES

A serious wilting and dieback of elms (*Ulmus* spp.) was first recorded in France in 1918, and subsequently spread throughout Europe rather rapidly. The disease and the causal agent, *Ophiostoma ulmi*, were fully described for the first time by Dutch researchers in 1922 (Holmes& Heybroek 1990).This first wave of Dutch elm disease in Europe had receded in importance by the mid-20th Century; although between 10–40% of infected trees were killed by this pathogen, many recovered. A more aggressive outbreak of the problem, however, had been recognized in North America in the early 1930s (Campana & Stipes 1981). A second species, *O. novo-ulmi*, was responsible for the outbreak in North America (Brasier 1991); this North America race of *O. novo-ulmi* (NAN) was inadvertently imported into Europe in the 1960s, causing a far more severe problem than the earlier outbreak of *O. ulmi*; almost all elms appeared to be highly susceptible to NAN. The situation in Europe is further complicated by the presence of a second race of *O. novo-ulmi* (EAN), which is spreading westwards from Eurasia (Brasier 1979).

The main trees affected by Dutch elm disease include all the European and American native elms: field elm (*Ulmus minor*), Wych-elm (*U. glabra*), European white elm (*U. laevis*), American elm (*U. americana*), red or slippery elm (*U. rubra*), rock elm (*U. thomasii*), and cedar elm (*U. crassifolia*) (http://www.eppo.org). Asian elm species are generally much less susceptible to the disease than are the Euro-American elms (Heybroek 1983). In Europe, spores of *O. ulmi* and *O. novo-ulmi* are vectored on the bodies of elm bark beetles in the genus *Scolytus* (*S. scolytus* and *S. multistriatus*); following mating, female *Scolytus* spp. excavate galleries beneath the bark of weakened trees and lay their eggs (Butin 1995). The microenvironment in the galleries is ideal for fungal sporulation; the pathogen produces coremia and perithecia into the galleries and, as the next generation of beetles emerge from pupation, spores adhere to their exoskeleton. Before mating, the newly emerged beetles must feed on the bark of living elms, usually on the twigs. Spores adhering to the beetle body are transferred to the wounds opened up by this maturation feeding and the tree becomes infected by the pathogen.

The fungus causes a vascular wilt that results in browning of the foliage and kills affected trees. This process can take anywhere between a few days to a few years, but with *O. novo-ulmi* is usually quite rapid. The fungus develops in the functional xylem, directly under the bark. The leaves of affected branches wilt, curl up and dry out, while turning yellowish or brownish, but usually remain attached to the tree. Cutting transversely through an infected branch reveals brownish vascular discoloration, due to the oxidation of phenolic compounds, in the affected xylem vessels.

Oak wilt, caused by *Ceratocystis fagacearum*, is one of the most destructive diseases of oaks in eastern North America. The main problem occurs on species in the red oak group, although many white oaks are also susceptible (Sinclair & Lyon 2005). Fortunately, this disease is not present in Europe (map 1). *C. fagacearum* appears to be indigenous to eastern and central North America (Kurdyla *et al.* 1995). The fungus spreads rather slowly, over short distances by root transmission through root grafts and common root systems shared between infected and healthy trees. Sap beetles in the family *Nitidulidae* are confirmed vectors of the pathogen and are responsible for long-distance transmission. International spread is possible through introduction of contaminated planting material or vectors inadvertently carried on oak products

Map1. Distribution of *Ceratocystis fagacearum, Phytophthora alni*

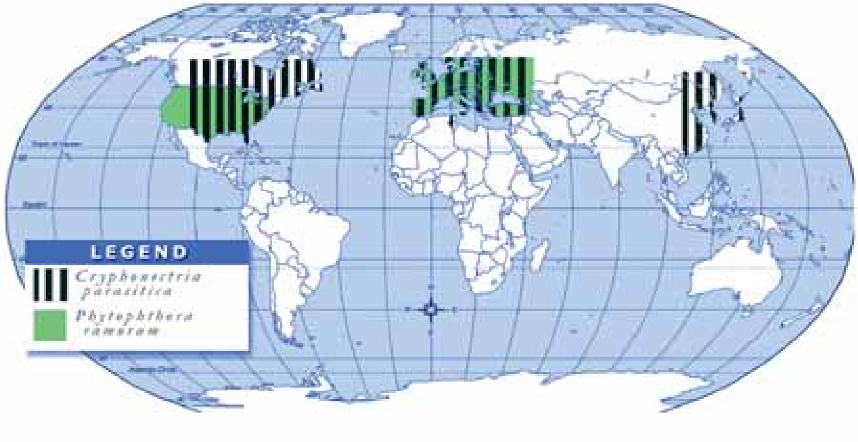
(Rexrode & Brown 1983). In infected trees, symptoms of the disease may occur as early as May. The leaves turn dull green or brown, appear water-soaked, wilt, and then turn yellow or brown. Damage occurs from the tip and outer edges toward the midrib and base. The disease progresses rapidly in red oaks, and some trees die within 1 or 2 months after the onset of symptoms. Most trees die within a year (French & Juzwik 1999). In contrast, white oaks may live for many years following infection.

Both the hybrid plane, *Platanus acerifolia* and the eastern European native oriental plane, *P. orientalis*, are susceptible to fatal attack by the canker stain pathogen, *Ceratocystis platani*. This pathogen is probably indigenous to the south-eastern USA and perhaps Mexico (Engelbrecht *et al.* 2004), whereas studies of the European populations of the pathogen suggest a single introduction from the USA to Naples, Italy during the Second World War (Ocasio-Morales *et al.* 2006). The disease is now present in Italy (Panconesi 1981), France (Ferrari & Pichenot 1974), Spain (Fernandez de Aña Magan & Gil 1977), Switzerland (Matasci & Gessler 1997), Greece (Tsopelas & Angelopoulos 2004) and there are unconfirmed reports from Serbia (Keca 2006, personal communication) and Belgium (Anselmi *et al.* 1994). *C. platani* is a wound parasite, colonizing exposed wounds immediately on contact and growing up to 2.0 – 2.5 m per year. Trees of 30–40 cm diameter can be killed within 2–3 years of initial infection (Panconesi 1999). Despite the common name of canker stain, the pathogen causes a wilting response in infected hosts, with tyloses blocking the vessels and resulting in a sudden wilting of a portion of the tree crown. Foliage turns yellow and wilts, later turning red-brown and remaining in the crown, being easily distinguished from the surrounding healthy leaves. Natural spread of the disease can be very slow. Healthy trees may be infected by root contact with a neighbouring diseased tree (Accordi 1986), or via natural wounds (weather damage, insect attack, rolling pebbles in watercourses). Ambrosia beetles are thought to be potential vectors of the disease. Wounds caused by human activities (pruning operations, road maintenance), however, are responsible for the majority of new infection cases in urban and rural environments. The disease is transferred to previously unaffected areas on infected planting material, or untreated packing and packaging material.

Losses in the urban populations of London plane in south-east France and Italy have been dramatic, seriously impacting on the aesthetics of these urban areas. Until recently the impact of *C. platani* on the Oriental plane appeared to be confined to Sicily, although the disease recently invaded Greece, entering the western limits of the natural distribution of Oriental plane, and causing the death of thousands of trees. Oriental plane is an important riparian species in the Greek flora; if the pathogen cannot be eradicated or controlled, further spread of the disease may lead to a huge ecological disaster. The pathogen is listed as an EPPO A2 quarantine pest (OEPP/EPPO 1986).

CANKER DISEASES

Cryphonectria parasitica was first reported in North America 1904 and during the next 50 years disease spread rapidly infecting trees of *Castanea* spp. (chestnut) all over the United States (Roane *et al.* 1986), causing chestnut blight. The outcome of the infection is death of the tree; in the USA, almost all chestnuts were killed by the 1930s,



Map 2. Distribution of *Phytophthora ramorum* and *Cryphonectria parasitica*

destroying industries reliant on the timber of these species. The disease was first noted in Europe in Italy in 1938 and now is present in France, Spain, Switzerland, the Western Balkans and Greece, and has spread into east Asia and Tunisia (Robin & Heiniger 2001) (map 2). The most common invasion pathway for *C. parasitica* is through international trade in host plants, timber and wood products. Conidia and ascospores of *C. parasitica* are spread in wind and rain, but are also transmitted by bark beetles (*Agrilus* spp.) and birds (Tainter & Baker 1996). The pathogen enters host trees via wounds produced by the insect vectors. On young, smooth-barked branches, blight-infected patches are bright brown, in contrast to the olive-green colour of normal bark. On older stem infections, the discoloration is less obvious (Bazzigher & Miller 1991).

PATHOGENS IN THE OOMYCOTA

In North America, sudden oak death (SOD) caused by *Phytophthora ramorum* attacked a range of species in the Fagaceae, and is of particular concern in California on *Lithocarpus densiflorus* (tanoak), *Quercus agrifolia* (coast live oak), *Q. kellogii* (black oak) and *Q. parvula* var. *shrevei* (Rizzo & Garbellotto 2003). Many other woody plants are also host to this destructive pathogen. In 1993, a serious dieback was noted on species and cultivars of *Rhododendron* and *Viburnum* in Germany and the Netherlands, with twig dieback, cankers, and leaf spots; *P. ramorum* proved to be the causal agent (Werres *et al.* 2001). By 2004, the pathogen was recorded in over 400 European woody plant nurseries and public gardens in 15 countries on 13 host genera. To date, *P. ramorum* has not been found in European native forest ecosystems. However, infected beech, horse chestnut, turkey oak, sweet chestnut, and Holm oak in woodland settings have been found at several sites in the UK. The most likely infection pathways are planting materials: wood, bark, and soil from areas where the disease occurs. As with other *Phytophthora* species attacking trees, infection by *P. ramorum* is often characterized by the presence of dark red to black sap oozing from the trunk ('bleeding cankers' or 'tarry spots'), usually on the lower stem, but sometimes occurring several metres above ground (http://www.the-tree.org.uk/TreeTalk/4/suddenoakdeath. htm#

What% 20are%20its%20symptoms). *P. ramorum* poses a large potential risk to European forest ecosystems, and was rapidly given quarantine status by the European Union, to monitor potential introductions from North America (http://www.eppo.org/QUARANTINE/Alert_List/fungi/oak_death.htm).However, *P. ramorum* isolates currently found in Europe are of a different mating type to that found in North America; should the two mating types be brought together, the problem caused by *P. ramorum* could markedly increase in severity.

The most widely distributed *Phytophthora* species, with nearly 1000 recorded host species, is *Phytophthora cinnamomi* (Zentmeyer 1980). First described attacking cinnamon trees in Indonesia (Sumatra) in 1922, the pathogen is now found all over the world, although its true origins remain unclear. *P. cinnamomi* is particularly destructive in the *Eucalyptus* forests of Western Australia and Victoria (Shearer & Tippett 1989). On a local scale, the pathogen is disseminated naturally by rain splash, by wind-blown soil or debris, or by water movement and run-off in drainage/irrigation ditches. The most likely means of more distant transport, however, is in contaminated soil or plant debris. Propagules can also be carried on machinery used for cultivation or harvesting and on seed. The first symptoms of infection by *P. cinnamomi* root rot are wilting and yellowing of foliage, which then dries out as the young feeder roots darken. Infected plants usually die from lack of water and nutrients, although there is considerable inter- and intra-specific variation in susceptibility to the disease.

Dieback and death of *Alnus* species, caused by *Phytophthora alni*, is present in many European countries: Austria, Denmark, France, Germany, Hungary, Italy, Netherlands, Sweden, UK, Belgium and Poland (Gibbs *et al.* 2003) (map 1). As with many other *Phytophthora* species attacking woody plants, *P. alni* causes lesions on the lower stem, root and collar necrosis and crown dieback. Alders are important species in substantial areas of wet natural woodland throughout Europe; the nitrogen-fixing ability results in greatly improved soil quality, particularly following regeneration in disturbed areas. Damage by *P. alni* has a significant ecological impact, including changes in forest composition, wildlife food and habitat, increased soil erosion and changes in soil composition. Nursery stock, including infected plants and contaminated soil, are the most likely means of long-distance transport of the pathogen. Wood with bark is another possible means for transport of the pathogen.

Many other species of *Phytophthora* are known from forest ecosystems outside Europe and may present significant threats if introduced. For example, *Phytophthora lateralis* kills both *Chamaecyparis lawsoniana* and *Pseudotsuga menziesii* in the Pacific North-West region of North America. Although also isolated from forests in France (Hansen & Delatour 1999), the pathogen has not caused serious problems in Europe to date. Moreover, there has been an rapid increase in the number of *Phytophthora* species recognized over the last 15 years, with 26 new species named; the damage potential of many of these species has yet to be determined.

INSECT PESTS

A particularly damaging and invasive insect in Europe is the horse chestnut leaf miner, *Cameraria ohridella*. This species was first recorded from Macedonia in 1985, attacking the common horse chestnut, *Aesculus hippocastanum* (Pavan *et al.* 2003).

Map 3. Known distribution of *Cameraria ohridella*

The current distribution of *C. ohridella* is shown in map 3. Based on the date of first record, the rate of spread for *C. ohridella* was estimated at 60 km/yr in Europe. This value is much higher than those calculated for other invasive organisms (Augustin *et al.* 2004). Leaf damage is particularly noticeable because horse chestnuts are abundant in towns and cities, and the defoliation of trees in streets, parks, and gardens occurs in early summer. At the country scale, the spread of *C. ohridella* has a stratified dispersal pattern with a positive association between the establishment of distant foci and human population density. It is likely that two mechanisms operate: long-distance dispersal by wind with a higher probability of establishment in cities because of the higher density of the host plant; and passive long-distance transport of *C. ohridella* between highly populated areas by vehicles (Kehrli & Bacher 2003).

CONCLUDING REMARKS

The importance of Oomycota in forestry has grown significantly in recent years. *Phytophthora citricola* dominates in nurseries being often found on ash, beech seedlings as well as silver firs. *P. cinnamomi* attacks Scots pine and *P. citrophthora* Norway spruce. *P. cambivora* has been found on dying adult beech, and may be implicated in decline and death of other broadleaved species. Alder decline is increasing in importance in many European countries because of the spread of *P. alni.* Newly emerged threats to forest ecosystems include *P. ramorum* and *P. kernoviae*. The danger of transferring *Ceratocystis fagacearum* from North America to Europe remains. A further factor to be considered is the possible hybridization between native and alien pathogens, which can give rise to stable hybrids causing problems on hitherto unaffected hosts (Brasier 2000, 2001). Devastation of *Aesculus hippocastanum* in Central Europe in recent years by the horse chestnut leaf miner, drew the attention of both public and environmentalists; the insect is still spreading northwards and westwards in Europe.

There are many questions that require addressing in the near future. Would *Ceratocystis fagacearum* be a serious danger for native European oaks? Will *Bursaphelenchus xylophilus* spread from its current limited range in Europe? What is the likely impact of climate change on the distribution of disease-causing agents such as

Cryphonectria parasitica, Ceratocystis platani, *Phytophthora* spp. and *Sphaeropsis pinea*?

These issues underline the importance of creating an international thematic network on Alien Invasive Species, in order to monitor spread of potentially damaging pests and pathogens. In this context, the new IUFRO working group provides an excellent international platform for discussion and information exchange among individuals and parties involved in this research.

References

Accordi S. M. 1986. Spread of *Ceratocystis fimbriata* f. *platani* through root anastomoses. Informatore Fitopatologico, 36: 53-58.

Anselmi N., Cardin L. & Nicolotti G. 1994. Plane decline in European and Mediterranean countries: associated pests and their interactions. EPPO Bulletin, 24: 159-171.

Augustin S., Guichard S., Svatos A., Gilbert M. 2004. Monitoring the regional spread of the invasive leafminer *Cameraria ohridella* (Lepidoptera: Gracillariidae) by damage assessment and pheromone trapping. Environmental Entomology, 33: 1584-1592.

Bazzigher G. & Miller, G. A. 1991. Blight-resistant chestnut selections of Switzerland: a valuable germ plasm resource. Plant Disease, 75: 5-9.

Blodgett J. T., Kruger E. L. & Stanosz G. R. 1997. *Sphaeropsis sapinea* and water stress in a red pine plantation in central Wisconsin. Phytopathology, 87: 429-434.

Brasier C. M. 1979. Dual origin of recent Dutch elm disease outbreaks in Europe. Nature, 281: 78-80.

Brasier C. M. 1991. *Ophiostoma novo-ulmi* sp. nov., causative agent of current Dutch elm disease pandemics. Mycopathologia, 115: 151-161.

Brasier C. M. 2000. The rise of the hybrid fungi. Nature, 405: 134-135.

Brasier C. M. 2001. Rapid evolution of plant pathogens via interspecific hybridisation. Bioscience, 51: 123-133.

Butin H. 1995. Tree Diseases and Disorders: Causes, Biology, and Control in Forest and Amenity Trees. Oxford University Press, Oxford, New York, Tokyo.

Campana R. J. & Stipes R. J. 1981. Dutch elm disease in North America with particular reference to Canada: Success or failure of conventional control methods. Canadian Journal of Plant Pathology, 3: 252-259.

Engelbrecht C. J. B., Harrington T. C, Steimel J. & Capretti P. 2004. Genetic variation in eastern North American and putatively introduced populations of *Ceratocystis fimbriata* f. *platani*. Molecular Ecology, 13: 2995-3005.

Fernandez de Aña Magan F. J. & Gil M. C. 1977. Estudio de las causas productoras de daños en la masa arbórea de la "Devesa" de Gerona. I.N.I.A. Departemento de Producción Forestal, Lourizan, Pontevedra, España.

Ferrari J. P. & Pichenot M. 1974. *Ceratocystis fimbriata* Ellis et Halsted f. *platani* (Walter), responsable d'une grave maladie du platane en France. La tache chancreuse. Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, D, 278: 2787-2789.

French D.W. & Juzwik J. 1999 Oak Wilt in Minnesota. http://www.extension.umn.edu/distribution/naturalresources/DD3174.html

Gibbs J. N., van Dijk C. & Webber J. F. (2003). *Phytophthora* disease of alder. Forestry Commission Bulletin, 126: 1-82.

Hansen E. & Delatour C. 1999. *Phytophthora* species in oak forests of north-east France. Annales des Sciences Forestière, 56: 539-547.

Heybroek H. M. 1983. Resistant elms for Europe. p. 108-113. [In:] Research on Dutch elm disease in Europe (Ed. A. Burdekin). Forestry Commission Bull. 60, London.

Holmes F. N. & Heybroek H. M. 1990. Dutch Elm Disease – The Early Papers. American Phytopathological Society Press, St. Paul, Minnesota.

Jung T., Blaschke H. & Osswald W. 2000. Involvement of soilborne *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. Plant Pathology, 49: 706-718.

Kehrli P. & Bacher S. 2003. Date of leaf litter removal to prevent emergence of *Cameraria ohridella* in the following spring. Entomologia Experimentalis et Applicata, 107: 159–162.

Kurdyla T. M., Guthrie P. A. I., McDonald B. A. & Appel D. N. 1995. RFLPs in mitochondrial and nuclear DNA indicate low levels of genetic diversity in the oak wilt pathogen *Ceratocystis fagacearum*. Current Genetics, 27: 373–378.

Matasci M. & Gessler C. 1997. Ein pilz bedroht die existenz der platane. Acta Veterinaria Hungarica, 45: 69-75.

OEPP/EPPO (1986). Data sheets on quarantine organisms No. 136, *Ceratocystis fimbriata* f.sp. *platani*. OEPP/EPPO Bulletin,16: 21-24.

Ocasio-Morales R. G., Tsopelas P. & Harrington T. C. 2006 Microsatellite analysis suggests that *Ceratocystis platani* (? *C. fimbriata f. platani*) was recently introduced to Greece from Italy. Proceedings of the Annual MSA-MSJ Meeting, Hilo, Hawaii 30 July – 4 August 2005 (in press).

Panconesi A. 1981. *Ceratocystis fimbriata* of plane trees in Italy: biological aspects and possibility of control. Proceedings of the Fifth Congress of the Mediterranean Phytopathological Union, Patras, Greece, 21-27 September 1980, 184-185.

Panconesi A. 1999. Canker stain of plane trees: a serious danger to urban plantings in Europe. Journal of Plant Pathology, 81: 3-15.

Pavan F., Barro P., Bernardinelli I., Gabon N., Zandigiacomo P. 2003 Cultural control of *Cameraria ohridella* on horsechestnut in urban areas by removing fallen leaves in autumn; Journal of Arboriculture, 29: 253-258.

Rexrode Ch. O. & Brown D. 1983 Oak Wilt Forest Insect & Disease. U.S. Department of Agriculture Forest Service Leaflet, 29. 5 pp.

Rizzo D. M. & Garbellotto M. 2003. Sudden oak death: endangering California and Oregon forest ecosystems. Frontiers in Ecology and the Environment, 1: 197-204.

Roane M. K., Griffin G. J. & Elkins J. R. 1986. Chestnut Blight, Other Endothia Diseases. and the Genus Endothia. American Phytopathological Society, St. Paul, Minnesota. 53 pp.

Robin C. & Heiniger 2001. Chestnut blight in Europe: diversity of *Cryphonectria parasitica*, hypovirulence and biocontrol. Forestry, Snow and Landscape Research, 76: 361-367.

Shearer B.L. & Tippett J. 1989. Jarrah dieback: the dynamics and management of *Phytopthora cinnamomi* in the jarrah (*Eucalyptus marginata*) forest of south-western Australia. Department of Conservation and Land Management Research Bulletin No. 3, Como, Western Australia.

Sinclair W. A. & Lyon H. H. 2005. Diseases of Trees and Shrubs. Cornell University Press, Ithaca, London. 2nd Edition.

Skilling D. D. 1977. The development of a more virulent strain of *Scleroderris lagerbergii* in New York State. European Journal of Forest Pathology, 7: 297-302.

Stanosz G. R., Trobaugh J., Guthmiller M. A. & Stanosz J. C. 2004 Sphaeropsis shoot blight and altered nutrition in red pine plantations treated with paper mill waste sludge. Forest Pathology, 34: 245-253.

Stephan B. R. & Schulze I. 1987. Scleroderris canker disease of conifers: bibliography of the disease, and its pathogen during the last 100 years (1883–1986). IUFRO Working Party S2.06.02. Bundesforschungsanstatlt fûr Forst und Holzwirtrschaft, Grosshansdorf, Germany.

"Sudden Oak Death" a new menace to trees in Britain 2004 http://www.thetree.org.uk/TreeTalk/4/suddenoakdeath.htm#What%20are%20its%20symptoms

Tainter F. H & Baker F. A. 1996. Principles of Forest Pathology. John Wiley, New York, Chichester.

Tsopelas P. & Angelopoulos A. 2004. First report of canker stain disease of plane trees, caused by *Ceratocystis fimbriata* f. sp. *platani* in Greece. Plant Pathology, 53: 531.

Werres S., Marwitz R., Man in't Veld W. A., De Cock A. W. A. M., Bonants P. J. M., De Weert M., Themann K., Ilieva E. & Bayen R. P. 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. Mycological Research, 105: 1155-1165.

http://www.eppo.org

http://www.eppo.org/QUARANTINE/Alert_List/fungi/oak_death.htm

Zentmeyer G. A. 1980. *Phytophthora cinnamomi* and the Diseases it Causes. Monograph 10, American Phytopathological Society, St. Paul Minnesota.

FORTHREATS: EUROPEAN NETWORK ON EMERGING DISEASES AND INVASIVE SPECIES THREATS TO EUROPEAN FOREST ECOSYSTEMS

Jan Stenlid[1], Alberto Santini[2], Andrea Vannini[3], Paolo Capretti[4],
Rimvis Vasiliauskas[1]

[1]Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, P.O. Box 7026, SE – 750 07 Uppsala, Sweden
[2]Istituto per la Protezione delle Piante C.N.R., Via Madonna del Piano I – 50019, Sesto fiorentino, Italy
[3]Dipartimento di Protezione delle Piante, Universita degli Studi della Tuscia, Via San Camillo de Lellis I – 01100, Viterbo, Italy
[4]Dipartimento di Biotecnologie Agrarie, Sezione di Patologia vegetale, Universita degli Studi di Firenze, Piazzale delle Cascine 28, I – 50144 Florence, Italy

INTRODUCTION

During February – March 2006 in Uppsala and Florence we initiated, elaborated and submitted to European Commission a proposal for the Coordination Action "European network on emerging diseases and invasive species threats to European Forest Ecosystems" (project acronym – "FORTHREATS"). The project falls within the Sixth Framework Programme, Priority 8.1 " Policy-oriented research – Scientific support to policies", and includes 23 partners and 12 associated participants from scientific institutions of 20 European countries, – Austria, Belgium, Czech Republic, Finland, France, Germany, Great Britain, Greece, Hungary, Italy, Latvia, Lithuania, Norway, Poland, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, and 6 external experts from Australia, China, Russia, South Africa and USA. In June 2006, FORTHREATS was approved by European Commission with a budget of 300 000 euros.

AIMS AND OBJECTIVES

The ultimate aims of FORTHREATS are to help preventing economic, ecological, social and cultural losses that emerging diseases and invasive species may cause to forests in Europe. Those aims will be achieved by setting up a network of experts, that also have contacts with other organisations, authorities, and research initiatives; by synthesising existing knowledge both published and available through the expert network; and by suggesting good practices and areas in need for further research or other attention.

The objectives of the project are to review the threats that are posed to European forests by organisms previously not present in the respective systems or organisms that have recently become invasive or causing emerging diseases. Organisms can either be imported from elsewhere, be present inside the area but make a host jump to come in contact with new hosts or originate from mutation or hybridization. Furthermore, due to changing environmental conditions, some pathosystems have developed into more critical stages due to host stress. The emerging diseases can either be of fungal, oomycete, bacterial or viral origin or be an insect or nematode. Invasive species can also be plants that are planted outside their normal geographical range and become invasive or that have otherwise spread into the European area. Of particular interest is to map the disease and threat situation in new member states of the European Union. A further aim is to identify any trade route or other import courts that require special attention.

We plan to make a synthesis of the literature findings, the outcomes of the partner meetings and discussions, and the input from the invited external experts. The synthesis will provide an overview of the threat situation in Europe broken down on geographic and climatic regions, and a rating of the degree of risk posed by the indicated threats. We will report on suggestions for good practices and standards for detection and monitoring. We also intend to identify undeveloped areas that require future research activities.

The specific objectives of the FORTHREATS project are: 1. Constitution of a network of expertise from old and new EU member states on possible threats to European forest ecosystems posed by emerging diseases and alien species. 2. Identifying and listing possible threats to European forest ecosystems posed by emerging diseases and alien species. 3. Identifying and listing likely origins of threats and modes of movement in Europe. 4. Qualifying and quantifying major threats in a geographic and climatic context. 5. Indicate i) possible ways for preventing introductions, ii) environmentally compatible control measures, and iii) suggested areas in need of attention for legislation. 6. Prepare suggestions for standardization of methodology. 7. Indicate areas where future research is needed.

STATE OF THE ART

Forests cover approx 46% of Europes 2.26 billion hectare land area which represent approx 27% of the total forested land area on earth (FAO, 2001). Forests provide raw material for economically important industries producing pulp, paper and timber etc. They are also important for biodiversity, for recreation and tourism and produce a multitude of ecosystem services e.g. function as carbon sinks thereby helping to buffer global warming. Maintaining forest ecosystem health and functioning is thus pivotal to a sustainable development, not only to the regions where forests dominate, but to the whole of the earth.

Introduced pathogens and pests have caused a number of international catastrophes to forest trees all over the world. The best known cases include Chestnut Blight that wiped out the American chestnut (Anagnostakis 1987), Dutch Elm Disease in both Europe and North America (Brasier 1991), White Pine Blister Rust on five-needle pines first in Europe and later in North America, *Phytophthora cinnamomi* on a high number of tree species in Australia and elsewhere (Hardham 2005), and Sudden Oak Death caused by *Phytophthora ramorum* in California (Rizzo and Garbelotto 2003). In Europe, the causative agent of the Sudden Oak Death was observed in 2001, and analyses of this disease have shown that other probably even more serious pathogens in European conditions have been observed both in Great Britain and Finland. Other examples with severe local impacts are *Serridium* cankers of C*upressus* sp (Graniti 1998) in southern Europe, *Certocystis fimbriata* on plane trees (Santini and Capretti 2000) and the recently reported *Eutypella* canker of *Acer* trees in Slovenia (Jurc et al. 2006). Other examples of emerging diseases have a less clear causal agent but may still be highly devastating, e.g. the current ash decline causing widespread dieback of ash trees in northern Europe.

One already well known example of a recent nematode invasion is the pine wood nematode (*Bursaphelenchus xylophilus*) vectored by *Monochamus* spp., which very recently was introduced to Portugal, but so far contained there successfully. This pest has the potential to severely affect the pine ecosystems in Europe and may also put quarantine restrictions on wood exports from infested areas. Insect examples include *Sirex noctilio* introduced from Southern Europe to Australia, New Zealand, South America and recently to South Africa, where, together with its fungal symbiont *Amylostereum aerolatum*, it has killed millions of hectares of pine plantations (Slippers et al. 2002) and the frequently intercepted *Anoplophora glabripennis* Asian long-horned beetle, which is under active eradication in Austria and Germany.

The origin of emerging diseases might also be associated with host jumps or new host ranges emerging as a result of species hybridisation (Brasier 2000, Slippers et al. 2005). One recent example is the hybrid *Phytophthora* species causing a massive dieback in European alder trees over the last decades (Brasier et al. 1999). The origin of the causal pathogen is hybridisation events between progenitor species that were not showing a virulent phenotype towards *Alnus* species, whereas the resulting hybrid is highly virulent and is currently spreading in the new host populations in Europe. Jumps between closely related host species have been reported to be associated with emerging diseases in *Eucalyptus* spp. in Australia and South Africa (Wingfield 2003; Burgess and Wingfield 2002). Threats caused by emerging diseases originating from hybridisation events or host jumps are likely to increase with the growing trade and movement of plant material. The enlarged European Union has also opened up for an increased movement of plant material making potential host-pathogen combinations possible.

Along with globalisation, the world trade of plant material including seedlings and timber can be expected to increase. Inspection of all plant material is conducted in order to restrict the migration of pathogens, but in order to be effective, information about the greatest potential risks should be available both for updating the quarantine lists and for the development of novel detection approaches based for example on molecular identification tools. Another area even more difficult to control is tourism, as tourists very often carry plants and seedlings as souvenirs; and with them potential threats. Such souvenirs do not usually go through inspection in customs.

Many exotic pathogens and pests may be highly virulent to European trees due to the lack of co-evolution between these threats and European hosts. Some organisms are likely to be relatively harmless to their host plant in their original dispersal area, but highly pathogenic to related species in the receiving country. However, the knowledge on the effects of the pathogens on the ecologically and economically important European tree species is insufficient and potential risks need to be assessed fully.

Overall, the history of introduced tree diseases and the changes expected during further globalisation can be anticipated to result in increasing pressure of pathogen invasions unless proper actions are taken. Proactive actions should be taken to identify and control pathogens and pests, not yet added to quarantine species lists. The first step to minimise the risk from yet unknown pathogens would be to identify the most serious threats in the relevant geographical regions, and then to focus the anticipatory action strategy on those pathogens.

Forestry-based economy is most important in northern Europe, where the low winter temperatures protect trees from many diseases. In the future, many pathogens and pests currently problematic in central Europe will move further north as climatic change proceeds. At the same time, northern Europe will also become more sensitive to those potentially dangerous pathogens in East Asia or North America, which do not survive in current low temperatures. Therefore, the combination of climate warming and globalisation of plant trade will set a previously unseen risk on the health of the European forests.

Forest trees and other plants have the potential to become invasive when grown as exotics, but it is difficult to predict which alien species are likely to cause serious damage if introduced. At present, the best guide to potential invasiveness is those species that have already caused problems when introduced into another part of the world. Thus, compilation of reliable information is critically important for assessing this risk. Protocols for assessing the risks of introductions should be developed, including forestry activities that may provide a pathway for the accidental introduction of alien species (FAO).

A number of organisations and network are already established e.g. World Conservation Union (IUCN) with its Invasive Species Specialist Group (ISSG), European Plant Protection Organisations (EPPO), and Delivering Alien Invasive Inventories for Europe (DAISIE) etc., that have the mission to describe and monitor new threats. However, none of these is specifically targeting the boreal and temperate forests present in Europe forest ecosystems or to identify new and emerging threats and research areas for monitoring and control. The present initiative is therefore highly motivated. There has been an EU-funded initiative BAWBILT resulting in a published book on the insect threats to European forest trees, although the emphasis was not on new and emerging pests. Specific disease threats have also been targeted with EU funding e.g. PHYTOFOREST and RAPRA for *Phytophthora* spp., POPFIME for population dynamics of Megastigmus insects on forest tree seeds, and PORT CHECK for technical development of detection methods of quarantine organisms. Our uptake is broader than these specific projects and we will include the findings of them in our synthesis. Other studies on threats to forests focus on abiotic stresses such as storms, fire or climate change have largely disregarded the biotic threats posed by emerging diseases and invasive species.

RELEVANCE TO SCIENTIFIC SUPPORT TO POLICIES WITHIN THE EU

This project addresses the objectives of the Scientific support to policies (SSP) Priority in a number of ways. The ultimate objective of the work is to provide underpinning scientific support for the formulation and implementation of additional and novel Community policies that target the prevention and restriction of entry of alien pathogens and pests into the European Community and wider European zone. Currently, there are many EU directives aimed at preventing and restricting entry of such organisms, but there is also a range of related policies within individual member states. The work carried out in this project will set the framework for standardisation of quarantine legislation and methodologies across the whole of Europe, strengthening current quarantine measures through the continent as a whole.

The work will be both timely and effective in providing scientific inputs to EU policy makers; the wide range of partners involved should enable the improved information generated to be exploited and taken up at national and EU levels. Moreover, the coherent research base provided by this work, integrating such a wide range of scientists and interested parties, reflects the increasing integration of Community policies with the underpinning science, fostering better relationships between researchers and policy makers within the EU.

The range of partners within the project, from EU and EFTA states, and from the international community represent a large pool of expertise in the various facets included in the project. The project strengthens the foundations of European research through: (a) bringing together a critical mass of tree pathologists, entomologists and quarantine specialists from with the EU and EFTA states; (b) including relevant experts from other continents where alien pathogens and pests either (potentially) originate or also threaten endemic forest ecosystems, and (c) includes associated partners and proposed guest speakers from SMEs involved in plant protection and quarantine implementation. In addition, the proposal to create long-term field sites for identifying potential threats within China will foster strong research links between EU states and that area. This integrated and coherent research will enable rapid progress in the work, which is an essential element in such activities.

The current reforms of CAP and the proposal to introduce new free trade policies may increase likelihood of transfer of alien pests within the EU, apart from the increasing threat of introduction of such organisms from outside the Community. Related to this is the threat of bioterrorism. EU crops, including all types of forest ecosystems, are potential targets for deliberate introductions of biological threats. The work proposed here is directed at precisely these threats, and the results will improve Europe's ability to detect such potential attacks in advance, thereby mitigating possible negative outcomes.

Outside the SSP, the project is of direct relevance to the thematic priority 7: Sustainable Development, focused on Global Change and Ecosystems. Forests, whether managed for timber production, or for environmental protection and amenity, are extremely important ecosystems within the rural setting. Protection against alien threats is of fundamental importance in maintaining our forestry base for issues as wide ranging as wildlife conservation, erosion and water catchment protection and rural employment. As highly important carbon sinks, forests represent crucial components in efforts to mitigate the impact of global climate change; potentially devastating alien pathogens and pests could have serious impacts on the ability of forests to act in this manner. Hence, the policy support arising from the work will further enhance attempts to improve the sustainable management of forest ecosystems. As experience in Europe and other continents has taught, alien pathogens and pests can cause severe disruption to forest ecosystems, resulting in severely degraded landscapes and depleted biodiversity.

In the context of productive forestry, the work will increase the ability of EU states to produce high quality timber, through the reduction in potential alien pathogen and pest attacks in future years. Such work will prevent potentially damaging bottlenecks arising within the forestry wood chain, protecting the industries reliant on timber, and as a consequence protecting many jobs within the rural sector, and also in the downstream processing sectors which represent some 10% of the EU workforce. Knowledge generated in this project will be utilized in the pursuit of novel management and technical methods to improve the detection, diagnosis. Further EU support may be sought to exploit innovation-related activities.

POTENTIAL IMPACT

The program deliveries include documents and a searchable database of best standard of schemes for detection and monitoring of threats posed by emerging diseases and invasive species. We will also point out areas in need of further attention. This includes both standardisation of detection methodology, monitoring, legislation and future research needs.

The Action will contribute to a detailed mapping of potential threats. Of particular importance is the fact that a large group of experts from old and new member states will be engaged in this process thereby contributing with knowledge and expertise from a wide range of different geographic and climatic settings. Complementary competences within the network will generate added value at the European level. Each expert will contribute with his/hers network of contacts with other scientists, practitioners and authorities in their home countries as well as internationally. In order to cover contacts with other continents we have also included experts from Australia, North America, Asia and Africa that both have top knowledge of the potential threats as well as experience of international legislation. We will also associate local experts to our meetings whenever appropriate.

The network that will be built up will also function for dissemination of knowledge. The partners and invited experts will feed back the information gathered in the program to their contacts in wider society. The added-value by the European dimension is through complementation of expertise, and awareness of threats from various regions. Also, the contacts created in the network are invaluable in future networking and expert interaction. Most partners are already highly involved in national and international committees on forest protection or quarantine issues such as EPPO. It will be the responsibility of the partners to inform local authorities on the results of the action. We will invite local authorities to the four meetings of the action.

A number of organisations and network are already established e.g. World Conservation Union (IUCN) with its Invasive Species Specialist Group (ISSG), European Plant Protection Organisations (EPPO), and Delivering Alien Invasive Inventories for Europe (DAISIE) etc that have the mission to describe and monitor new threats. In order to facilitate information exchange and data mining, we have included in our consortium experts that are associated with these organisations. There are also links to other EU funded research programs e.g. BAWBILT, PHYTOFOREST and RAPRA. Contacts with these organisations will also work for our information dissemination activities by providing already built-up networks for contact and extension.

By including experts from many regions in Europe, the network will make the partners and associated contributors more aware of potential threats that might move into the various countries. Authorities and companies will be invited to the meetings locally. The action will set up a homepage where documents and results of the activities will be made available to the general public. The dissemination plan will be facilitated by compiling lists that will be available in printed versions as well as electronically on the home page. The searchable database of standard identification, good monitoring practices, and key literature references will become a valuable platform for communication.

PROJECT MANAGEMENT AND DISSEMINATION PLANS

The program will be lead by the co-ordinator Jan Stenlid. There is a co-ordination committee with special responsibility for the summary and reporting work. This group consists of Alberto Santini, Andrea Vannini, Steve Woodward, Thomas Kirisits, Rimvis Vasiliauskas, and Jan Stenlid as the leader. The program is organised into a network with 23 partners with 12 associated participants. We have invited six external experts to take part in the meetings and exchange of information from South Africa, Australia, China, Russia and USA. In order to facilitate the contacts and discussions, project website will be produced.

We defined objectives of the network as:

A. Constitution of network of expertise of possible threats to European forest ecosystems posed by emerging diseases and alien species.

B. Identifying and listing of possible threats to European forest ecosystems posed by emerging diseases and alien species.

C. Identifying origins of threats and modes of movement in Europe.

D. Qualifying and quantifying threats in a geographic and climatic context.

E. Networking to reach standardization of methodology.

F. Syntesis and reporting.

These objectives are reflected in the five work packages (WP); A. Diagnosis and monitoring (Responsible partner A. Vannini) B. Origin (Responsible A. Santini), C. Spread (Responsible J. Stenlid) D. Impact (Responsible S. Woodward) E. Synthesis and reporting (Responsible J. Stenlid).

Decisions on critical issues will be taken by the Co-ordinator in consultation with the co-ordination committee. Possible risks in achieving the project objectives are minimised by a high number of participants. In other words, the information on emerging diseases and alien species will be retrieved from many partners, often from the neighbouring regions, and at the same time covering all geographic areas of Europe. Thus, the likelihood of presenting the relevant and comprehensive data on European scale (and achieving project objectives) is very high even in cases where some partners will encounter obstacles to deliver the required data.

We plan for 24 months duration and 4 meetings two of which may be in conjunction with international conferences, and 2 weeks of working time per partner keeping in mind that the maximum contribution from EU at this time is only 0.3 million euros. The budget for travel expenses of the associated participants and the external experts to visit our meetings is retained and coordinated by the co-ordinator who will reimbursed costs. For partners, the travel costs to 4 meetings each are distributed among the partners. One extra meeting is planned for the final synthesis by the co-ordination committee. In order to run this network, six man months of work is set aside in the budget of the co-ordinator.

The results of this action will be made available to the general public in form of published lists and searchable databases. Whenever possible, the results will be published in international scientific journals. Intellectual property rights will be kept with the researchers according to the local legislation.

References

Anagnostakis S. L. 1987. Chestnut blight – the classical problem of an introduced pathogen. Mycologia, 79: 23-37.

Brasier C. M. 1991. *Ophiostoma novo-ulmi* Sp-Nov, causative agent of current Dutch elm disease pandemics. Mycopathologia, 115: 151-161.

Brasier C. M. 2000. Plant pathology – The rise of the hybrid fungi. Nature, 405: 134.

Brasier C. M., Cooke D. E. L., Duncan J. M. 1999. Origin of a new *Phytophthora* pathogen through interspecific hybridization. PNAS: 5878-5883.

Burgess T, Wingfield M. J. 2002. Impact of fungi in natural forest ecosystems; A focus on *Eucalyptus.* [In:] Microorganisms in Plant Conservation and Biodiversity (eds K. Sivasithamparam, K. W. Dixon, R. L. Barrett). Dordrecht, Kluwer Academic Publishers, pp. 285-306.

Graniti A. 1998. Cypress canker: A pandemic in progress. Annual Review of Phytopathology, 36: 91-114.

Hardham A. R. 2005. *Phytophthora cinnamomi*. Molecular Plant Pathology, 6: 589-604.

Jurc D., Ogris N., Slippers B., Stenlid J. 2006. First report of *Eutypella* canker of Maple in Europe. Plant Pathology, (In press).

Rizzo D. M., Garbelotto M. 2003. Sudden oak death: endangering California and Oregon forest ecosystems. Frontiers in Ecology and the Environment, 1: 197-204.

Santini A, Capretti P. 2000. Analysis of the Italian population of *Ceratocystis fimbriata* f.sp *platani* using RAPD and minisatellite markers. Plant Pathology, 49: 461-467.

Slippers B, Wingfield B. D., Coutinho T. A. Wingfield M. J. 2002. DNA sequence and RFLP data reflect relationships between Amylostereum species and their associated wood wasp vectors. Molecular Ecology, 11: 1845-1854.

Slippers B., Stenlid J., Wingfield M. J. 2005. Emerging pathogens: fungal host jumps following anthropogenic introduction. Trends in Evolution and Ecology, 20: 420-421.

Wingfield M. J. 2003. Increasing threat of diseases to exotic plantation forests in the Southern Hemisphere: lessons from *Cryphonectria* canker. Australasian Plant Pathology, 32: 133.

OLD AND NEW PATHWAYS FOR INVASION OF EXOTIC FOREST INSECTS IN EUROPE

FIRST RESULTS OF THE EUROPEAN PROJECT DAISIE

Alain Roques

INRA, UR633 Zoologie Forestičre, F-45166 Olivet, France,
e-mail: alain.roques@orleans.inra.fr

INTRODUCTION

Biological invasions by alien species are presently recognised as the second cause of loss in biological diversity, following the destruction of habitats, and have also large economical consequences (Vitousek *et al.* 1996, 1997; Wilcove *et al.* 1998; Mack *et al.* 2000; Perings *et al.* 2000; Pimentel *et al.* 2000). This is especially true for forestry because forest products are moved around the globe with ever-increasing speed and frequency as a result of technological advances and of globalisation trade. This movement has precipitated a substantial increase in biological invasions by allowing organisms to pass the natural barriers that limit their dispersal (Liebhold *et al.* 1995; Levine and D'Antonio 2003). Forest pests can move between countries via a number of pathways including importation of logs, chips, solid wood packaging and propagative materials (for a review, see a CD-Rom archive of the Internet forum "Risks of Exotic Forest Pests and their Impact on Trade"; The American Phytopathogical Society 2001; McCullough *et al.* 2006). Thus, during the past century the introduction of exotic forest pests into highly vulnerable tree populations has resulted in severe ecologic, aesthetic and economic impacts. For example, in North America the European gypsy moth (*Lymantria dispar* [L.]), balsam woolly adelgid (*Adelges piceae* [Ratzeburg]), pine shoot moth (*Rhyacionia buoliana* [Denis & Schiff.], and more recently Asian long-horned beetle (*Anoplophora glabripennis* [Motsch]) and emerald ash borer (*Agrilus planipennis* Fairmaire) caused irreparable damage to forested ecosystems and landscape trees (Mattson 1997, Britton and Sun 2002; Haack *et al.* 2002), as did introduced tree diseases such as chestnut blight (*Cryphonectria parasitica* [Murr.] Barr.) (Schumann 1991). In 1994, Mattson *et al.* (1994) estimated that there were more than 368 alien phytophagous species in wooded areas of America north of Mexico.

In contrast, insect invasions in forests are much less documented in Europe. No list of alien insect species was available in any of the European countries until 2002. Then, such lists were successively supplied for Austria (Essl and Rabitsch 2002), Germany (Geiter *et al.* 2002), Switzerland (Kenis 2005), the Czech Republic (Šefrová and Laštùvka 2005), and Scandinavia (www.nobanis.com, 2005), but usually without specific information on alien forest insects, their pathways of introduction and their hosts. Moreover, no global list of alien forest insects yet exists at the European level.

The recent development of two European research projects, which both aim at understanding the development of biological invasions in Europe, is expected to modify significantly this situation. The project "ALARM" (*Assessing Large-scale Risks with tested Methods*, 2004–2009) mainly aims at developing standardized risk assessment methods for invasions whilst a major objective of "DAISIE" (*Delivering Alien Invasive Species Inventories in Europe*, 2005–2008) consists in supplying a European database of alien organisms, quantified country by country. The large involvement of forest entomologists within these two projects leads to expect that a specific European database for alien forest insects will be soon obtained including most of the information available about origin, hosts, introduction pathways, and impact of these species. A preliminary analysis developed within these projects consisted of synthesizing the notifications of non-compliance regularly published by the European and Mediterranean Plant Protection Organization (EPPO) from the reports of alien species interceptions conducted by the National Services of Inspection and Quarantine at ports-of-entry and country borders. Such interception records could be valuable for the prediction of invaders and for our general understanding of invasions. Brockerhoff *et al.* (2006) showed that species of bark and ambrosia beetles frequently intercepted in New Zealand were about four times as likely as rarely intercepted species to be established somewhere.

The objectives of this paper are first to present the synthesis of the EPPO interception data for forest insects introduced in Europe. Then, the first, preliminary results obtained through the project DAISIE for the establishment of alien forest insects in Europe will be detailed in order to determine whether interception rates are related to establishments.

ANALYSIS OF THE INTERCEPTIONS OF ALIEN FOREST INSECTS ARRIVING IN EUROPE

Data were extracted from the «Reporting Services», which are bulletins regularly published (usually 12 times per year) by EPPO, where notifications of non-compliance (detection of regulated pests) are reported country by country for the EPPO. The analysis considered all the bulletins published since issue 1996-1 (January 1996), reporting pest interceptions having occurred in 1995, until 2005-5 (August 2005), reporting pest interceptions having occurred in 2004. Only the interceptions concerning Europe in a geographic sense were considered. According to the availability of data, a total of 29 countries were thus concerned: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxemburg, Malta, the Netherlands, Norway, Poland, Portugal, Romania, Slovenia, Spain, Sweden, Switzerland, and the United Kingdom. We removed from the interception lists all the species of European origin to consider only the non- indigenous, alien species defined following Pyšek *et al.* (2004) as "*taxa in a given area whose*

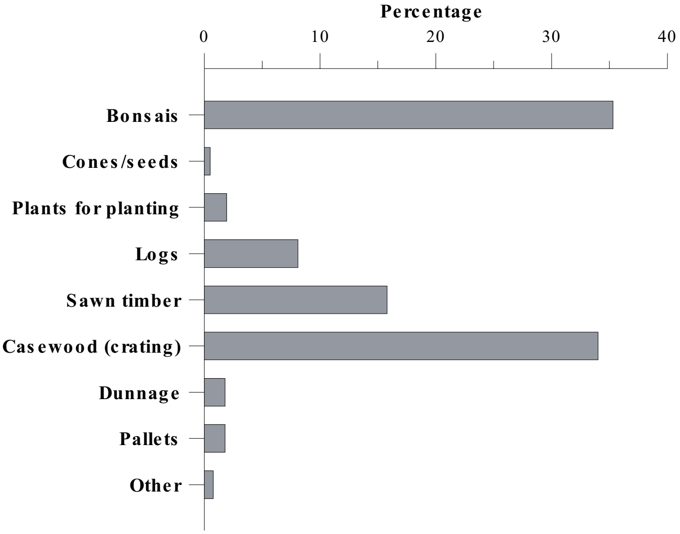


Figure 1. Relative importance of the different categories of commodities associated with interceptions of alien forest insects in Europe from 1995 to 2004

presence there is due to intentional or unintentional human involvement, or which have arrived there without the help of people from an area in which they are alien". However, cryptogenic species (i.e., species of unknown origin such as cosmopolitan pests of stored products) were included.

We first surveyed the following variables: (1) relative importance of associated commodities classified into the following nine categories: bonsais, plants for planting, seeds, logs, sawn timber, casewood (crating), dunnage, pallets, and miscelleanous; and (2) origin, classified into the following regions: Asia, Australasia, Europe, Russia, North Africa, Southern Africa, Tropical Africa, North America, and South America.

Of the 6745 insect interceptions having occurred between 1995 and 2004 (Roques and Auger-Rozenberg 2006), 779 concerned insects related to forest and ornamental trees. However, only 281 specimens were identified at the species level (i.e., 36.0%) for a total of 42 species. Usually, larvae were identified at family level whilst a lot of interceptions only mentioned "grub holes larger than 3mm", i.e., probably due to cerambycids. This major problem of identification prevented any definite conclusion about the specific rate of arrival of alien species. Work *et al.* (2005) additionally pointed out that the estimation of arrival rates cannot be obtained in the absence of negative interception records. However, some general information could be inferred from these data. The interception of alien forest insects was much more important on wood packaging material (37.6%), and especially casewood (34.0%), than on sawn timber (15.8%) and logs (8.1%) (Figure 1). However, the trade of bonsais played a quite equivalent role in providing 35.3% of the interceptions. The bonsai trade is known to be responsible for the introduction of at least one major tree pest in France, Italy and USA in recent years: the citrus longhorned beetle, *Anoplophora chinensis* (Hérard *et al.* 2005).

The origin of alien insects differed significantly depending on the associated commodity (χ^2 test; P=0.000; Figure 2). The insect species arriving with bonsais and wood

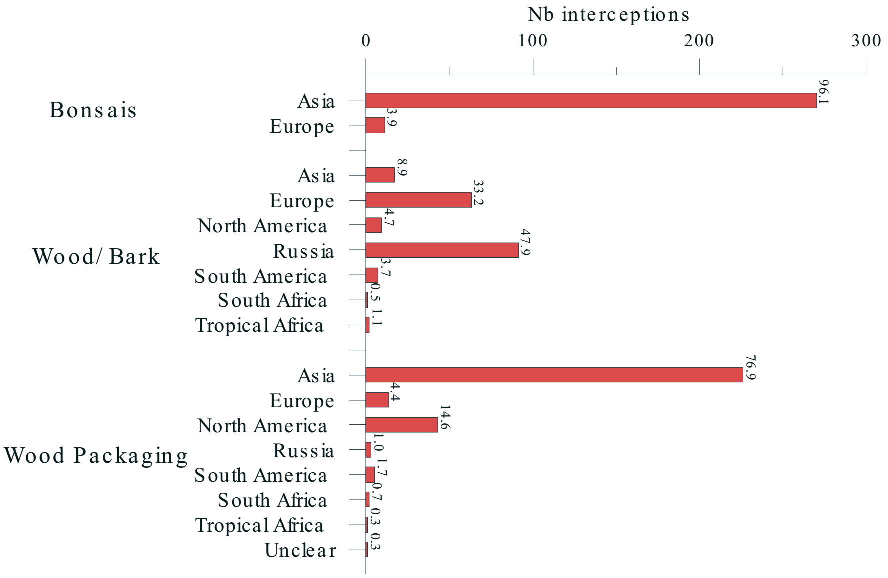


Figure 2. Geographic origin of the alien forest insects intercepted with plant and wood trade from 1995 to 2004 in Europe

packaging material originated very predominantly from Asia (96.7 and 76.9%, respectively) whereas thouse arriving with timber and logs came from Russia (47.9%) but also through intra-European exchanges. In addition, the diversity of alien insects carried by bonsais was significantly higher than that of the entomofauna carried by wood packaging material and fresh wood (χ^2 test; P=0.000; Figure 3). Small sap-feeding insects such as aphids, scales and psyllids were dominant in bonsais whereas wood packaging and fresh wood mostly hosted coleopteran species, especially long-horned beetles together with bark beetles in fresh wood.

Preliminary analysis results from DAISIE: Alien forest insects established in Europe

As indicated previously, aliens were defined as the species whose presence in a European country resulted directly or indirectly from human activity. Therefore, all native species naturally expanding in Europe, e.g., with global warming, such as the pine processionary moth were excluded. Three categories of aliens were considered: (i) exotic species originating from other continents; (ii) species of European origin introduced in a country where it could be ascertained that they were originally absent; (iii) cosmopolitan species whose origin was unknown in absence of genetic studies ("cryptogenic" species). The second category was especially important because of the existence in Europe of a large number of islands where insects from continental Europe were introduced together with their host trees (e.g., most conifer-related species in the United Kingdom). Because the DAISIE project lists are still in the process of validation by experts for each of the taxonomic groups, the numbers and percentages given below

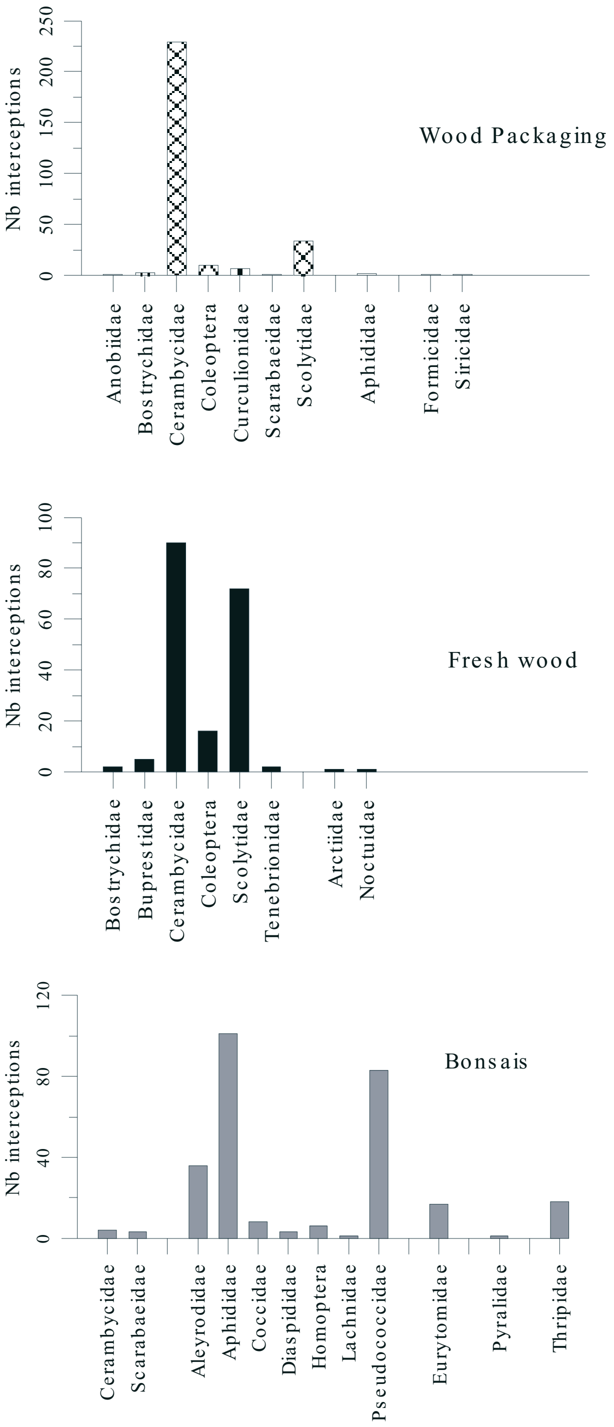


Figure 3. Relative importance of the different insect families in the total number of alien forest insects intercepted with bonsais, wood packaging material and fresh wood in Europe from 1995 to 2004

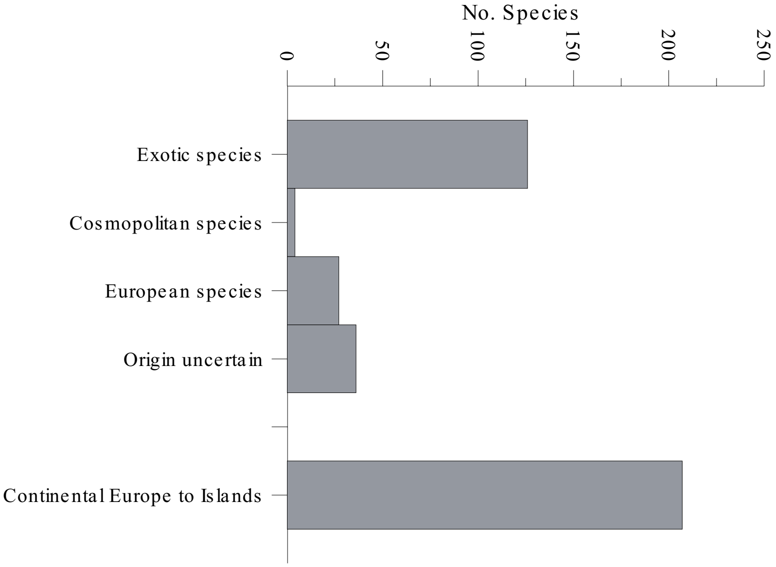


Figure 4. Relative importance of the different categories of alien forest insects established in Europe

must be considered as preliminary and taken with caution. However, the trends shown by these data seemed relatively stable.

Of the 1401 species considered for the moment as aliens in a European country, 193 are related to forest and ornamental trees. If we exclude the species from continental Europe introduced into islands (especially represented by the United Kindgom, and much less by Corsica, Sicily, Malta, and the Canary islands), the major part of the alien species are of exotic origin (Figure 4). Although some data about the period of introduction are still missing (origin uncertain in 26.4% of the cases), it is likely that establishment in Europe of alien forest insects accelerated considerably during the second half of the 20th century, and especially since the 1990s (Figure 5). At least 42.4% of the alien species arrived in Europe between 1950 and 2006. The largest proportion of these species originated from Asia (21.9%; Figure 6), and more especially from China, followed by a little less from North America (19.8%) but the proportion of established species originating from Australasia was surprisingly important, probably in relation with the introduction of several economically-important tree species (e.g., *Eucalyptus* ssp., *Acacia* spp.) from this region.

Virtually all (>98%) the established alien insect species resulted from non- intentional introductions. Most (>75%) came as plant contaminants whereas a few (<10%) travelled as hitchhikers (e.g., the horse chestnut leaf miner, *Cameraria ohridella*, and several ant species). Less than 2% proceeded from deliberate human activities such as biological control or leisure (e.g., saturnid moths).

A comparison of the interceptions and establishment of alien species for three important groups of forest insects, Cerambyidae, Scolytidae, and Lepidoptera is presented in Figure 7. It appeared that the major part (>80%) of the established species were never intercepted; e.g. the cerambycid *Neoclytus acuminatus*. The same was true for pest insects of other families, e.g. the platypodid *Platypus mutatus* and the oriental chestnut

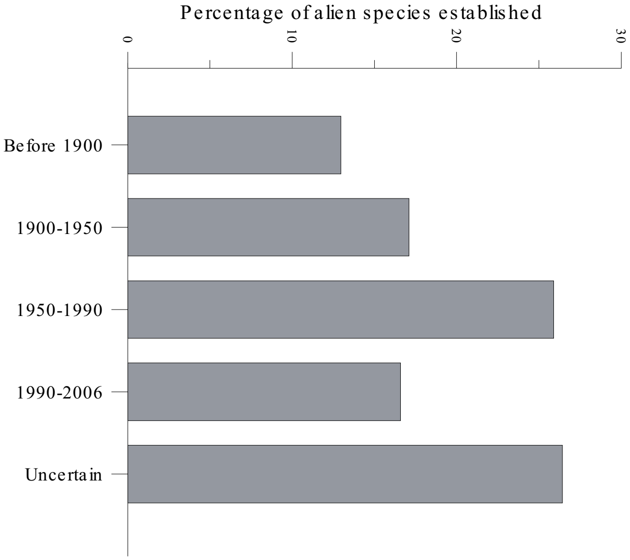


Figure 5. Date of arrival in Europe of alien forest insects which are presently established (Islands are not included in the calculations)

gall wasp, *Dryocosmus kuriphilus* which were recently observed in Italy. In contrast, several intercepted species never established such as Asian cerambycids of the genera *Batocera* and *Apriona*, and north American bark beetles of the genera *Ips* and *Dendroctonus*.

Indeed, several factors prevented any reliable comparison between established alien species and the intercepted ones. The detection efforts probably differed considerably between countries but, in any event, they were usually limited to targeted commodities. Inspection priorities may have also varied depending on pests of current

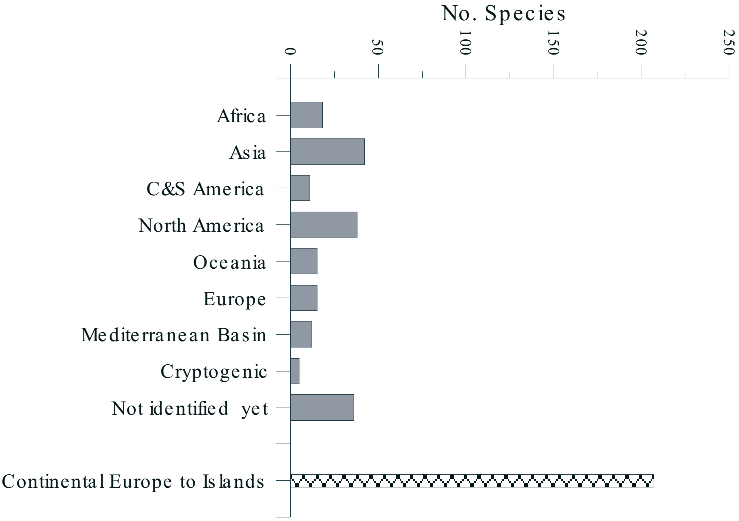


Figure 6. Geographic origin of the alien forest insects established in Europe

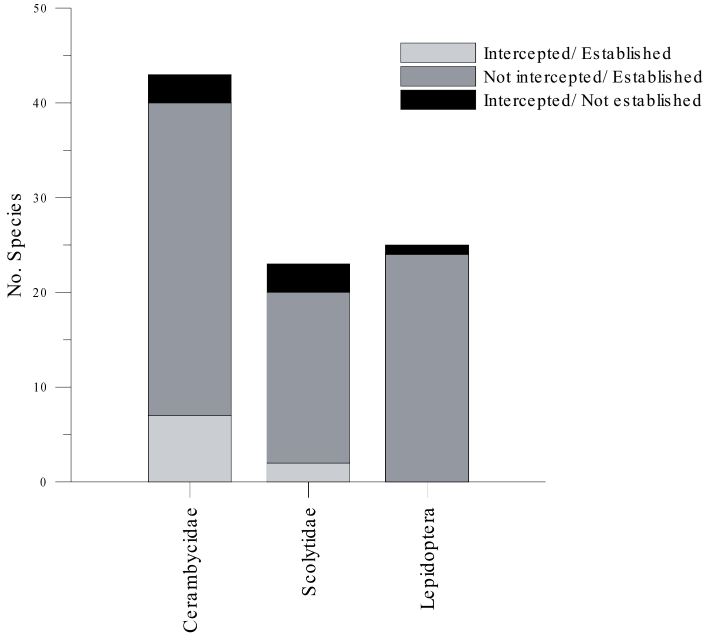


Figure 7. Comparison between intercepted and established alien species of forest insects in the family Cerambycidae and Scolytidae and in the order Lepidoptera in Europe

concern but did not consider all the potentially- present NIS. Analysing four cargo pathways, Work *et al.* (2005) estimated that inspections probably detected only 19% to 50% of the species being transported, depending on the particular pathway. Finally, it would be worthwhile to consider in detail all the established alien species that have never been intercepted in order to identify the commodities possibly associated with them and the possible pathways of invasion, and to suggest adapted measures of prevention.

CONCLUSIONS

Attention must be focused on new pathways such as bonsai trade which allows small sap-feeding insects as well as large xylophagous larvae to travel inconspicuously with their host plants. However, discrepancies observed between records of established and intercepted alien species indicate that other, as yet unidentified pathways of invasion may exist for alien forest insects. The completion of the DAISIE project by late 2007 is expected to help in identifying these pathways.

Acknowledgements. *We thank very much the EPPO Staff, and especially Françoise Petter, Andreï Orlinski, and Anne-Sophie Roy, for providing the data and permitting their use in this analysis. This work was funded by the European Commission within the FP6 Integrated Project "ALARM" (Assessing large – scale environmental risks with tested methods- GOCE-CT-2003-506675) and the FP6 Specific Target Research Project "DAISIE" (Delivering alien Invasive Species Inventories for Europe- SSPI-CT-2003-511202).*

References

Britton O. K., Sun J. H. 2002. Unwelcome guests: Exotic forest pests. Acta Entomologica Sinica, 45: 121-130.

Brockerhoff E. G., Bain J., Kimberley M., Knížek, M. 2006. Interception frequency of exotic bark and ambrosia beetles (Coleoptera: Scolytinae) and relationship with establishment in New Zealand and worldwide. Can. J. For. Res., 36: 289–298.

Essl F., Rabitsch W. 2002. Neobiota in Österreich. Umweltbundesamt, Wien, 432 pp.

Geiter O., Homma S., Kinzelbach R. 2002. Bestandsaufnahme und Bewertung von Neozoen in Deutschland. Umweltforschungsplan des Bundesministeriums für Umwelt, Naturschutz und Reaktorsicherheit. Forschungsbericht 296 89 901/'1 UBA-FB 000215.

Haack R. A., Jendek E., Liu H., Marchant K.R., Petrice T. R., Poland T. M., Ye H. 2002. The emerald ash borer: a new exotic f orest pest in North America. Newsl. Mich. Entomol. Soc., 47: 1–5.

Hérard F., Jollivet C., Reynaud P. 2005. Gestion des foyers de capricornes asiatiques: en France, Autriche, Allemagne et Italie, l'éradication est lancée. Phytoma, 583: 22-26.

Kenis M. 2005. Insects-Insecta. [In:] An inventory of alien species and their threat to biodiversity and economy in Switzerland (ed. R. Wittenberg). CABI Bioscience Switzerland Centre. Centre report to the Swiss Agency for Environment, Forests and Landscape, pp. 131-212.

Levine J. M., D'Antonio C. M. 2003. Forecasting biological invasions with increasing international trade. Conservation Biology, 17: 322–326.

Liebhold A. M., MacDonald W. L., Bergdahl D., Mastro V. C. 1995. Invasion by exotic forest pests: A threat to forest ecosystems. Forest Sciences Monographs, 30:1-49.

Mack R. N., Simberloff D., Lonsdale W. M., Evans H., Clout M., Bazzaz F. A. 2000. Biotic invasions: Causes, epidemiology, global consequences and control. Ecological Applications, 10: 689-710.

Mattson W. J. 1997. Exotic insects in North American forests: Ecological systems forever altered. [In:] Proceedings of the Conference on Exotic Pests of Eastern Forests (ed. K. O. Britton). USDA Forest Service and Tennessee Exotic Pest Plant Council, Nashville, Tennessee, pp. 187-193.

Mattson W. J., Niemelä P., Millers I., Inguanzo Y. 1994. Immigrant phytophagous insects on woody plants in the United States and Canada: An annotated list. General Technical Report NC-169. St Paul (MN), US Department of Agriculture.

McCullough D. G., Work T. T., Marshall D., Cavey J. F., Liebhold A. 2006. Interceptions of nonindigenous plant pests at U.S. Ports of entry and border crossings over a 17-year period. Biological invasions, 8: 611-630.

Perings C., Williamson M. , Dalmozzone S. 2000. The economics of biological invasions. Edward Elgar, Cheltenham, United Kingdom.

Pimentel D., Lach L., Zuniga R. Morrison D. 2000. Enviromental and economic costs of nonindigenous species in the United States. Bioscience, 50: 53–65.

Pyšek P., Richardson D. M., Rejmánek M., Webster D.L., Williamson M., Kirschner J. 2004. Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. Taxon, 53: 131- 143.

Rabitsch W., Essl F. 2005. Biological invasions in Austria: patterns and case studies. Biological invasions, 8: 295-308.

Roques A., Auger- Rozenberg M. A., 2006. Tentative analysis of the interceptions of nonindigenous organisms in Europe during 1995–2004. EPPO Bulletin, in press.

Schumann G. L. 1991. Plant diseases: Their biology and social impact. APS Press, St. Paul, Minnesota.

Šefrová H., Laštùvka Z. 2005. Catalogue of alien animal species in the Czech Republic. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 53: 151-170.

Vitousek P. M., D'Antonio C. M., Loope L. L., Rejmanek M. Westbrooks R. 1997. Introduced species: a significant component of human-caused global change. New Zealand Journal of Ecology, 21, 1- 16.

Vitousek P. M., D'Antonio C. M., Loope L. L., Westbrooks R. 1996. Biological invasions as global environment change. American Scientist, 84: 468-478.

Wilcove D. S., Rothstein D., Dubow J., Phillips A., Loso E. 1998. Quantifying threats to imperiled species in the United States. Bioscience, 48: 607–615.

Work T. T., McCullough D. G., Cavey J. F., Komsa R. 2005. Arrival rate of nonindigenous insect species into the United States through foreign trade. Biological Invasions, 7: 323-332.

SPATIOTEMPORAL DYNAMICS OF INTRODUCED BARK BEETLES

Bjørn Økland[1], Olav Skarpaas[2], Kyrre Kausrud[3], Nils Chr. Stenseth[3],
Nadir Erbilgin[4]

[1]Norwegian Forest and Landscape Institute, P.O.Box 115, N-1430 Ås, Norway
[2]Norwegian Institute for Nature Research, Gaustadalleen 21, NO-0349 Oslo, Norway
[3]Centre for Ecological and Evolutionary Synthesis, Department of Biology,
University of Oslo, P.O.Box 1050 Blindern, N-0316 Oslo, Norway
[4]Nadir Erbilgin,140 Mulford Hall, Division of Organisms and Environment,
University of California, Berkeley CA 94720

Biological invasions cause serious ecological and economic impacts around the world (D'Antonio and Kark 2002, McGrath and Farlow 2005, Pimentell 2002). Once alien pest species have become established in their new habitats, they may be extremely difficult to eradicate, and the costs of damages and control programmes may be very high. In many cases the damage caused by the invading organisms is more serious in the new range than in the native range of the pest (e.g. *Dendroctonus valens* in China). In Scandinavian forests, the native spruce bark beetle *Ips typographus* causes significant economic damage on Norway spruce. The last outbreak of this species in Norway (1971–1981) killed the equivalent of 5 million m^3 of spruce timber within a 140,000 km^2 area (Bakke 1989).

A few years ago, a close relative of *I. typographus*, the non-native eight-toothed spruce bark beetle (*Ips amitinus*), was found in bark samples from imported Russian and Baltic timber (Økland 2002, Thunes *et al.* 2003). More recently, the first hibernating individuals of *I. amitinus* were trapped at a timber storage site in Southern Norway, in which large amounts of Baltic timber had been stored up to 2003 (Fig. 1). However, all of the timber was removed in 2004 when we conducted a trapping using synthetic pheromones in a gradient from the storage centre and out to the surrounding forest (Økland *et al.* 2005). We used *I. typographus* pheromones in the traps, because *I. amitinus* attractants (Amitinuswit) were not delivered prior to the major flight season of this pest. We used pheromones of *I. typographus* because it consists of 3 compounds, *S-cis*-verbenol, 2-methyl-3-buten-2-ol and ipsdienol and some of these compounds, such as ipsdeinol, may be attractive to *I. amitinus*. Even with this less effective attractant, we captured three individuals of *I. amitinus*, which most probably emerged from their hibernation in the ground below the timber. We suspect that the density *I. amitinus* in the traps would probably have been much higher with a specific pheromone for this species. During the swarming season, the total density of all bark beetles (mainly *I.*

Figure 1. The first records of hibernating *Ips amitinus* in Scandinavia were done in this timber storage in southern Norway. Note the close distance to surrounding forests

typographus) was about an outbreak level in the central traps and declined with distancet *al.* 2005); however, other intermediate timber storages are still in use.

Knowledge about the spread of new bark beetle species is critical because of their ecological and economic impacts on native renewable resources. Aggressive bark beetles are some of the most destructive insects in temperate conifer forests and kill virtually all susceptible host trees over extensive areas during outbreaks. Many of these species are not currently present in Scandinavia, such as *Dendroctonus ponderosae* and *D. frontalis* that killed pines equivalent to about 50 million m^3 in the period 1979–1983 in the USA (Hoffard 1985, McGregor 1985). Other harmful bark beetle species, such as *D. rufipennis* and *I. pertubatus* in Alaska, could potentially amplify the outbreaks of *I. typographus* if they were introduced into Scandinavian spruce forests (Bright 1976, Furniss and Carolin 1977, Werner and Holsten 1997, Wood and Van Sickle 1992,

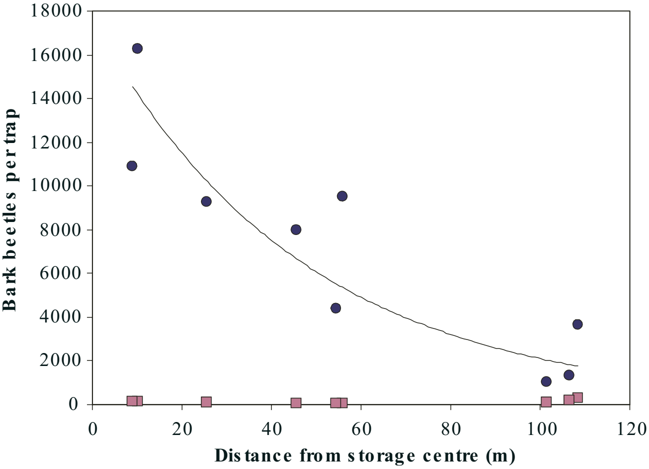


Figure 2. Density of bark beetles per trap plotted against distance from the timber storage centre. Trapping periods were 22 April – 14 May (circles) and 14 May – 13 July (squares). Modified figure from Økland *et al.* 2005

Wood 1982). Another bark beetle species, *I. cembrae*, is known as an aggressive and damaging species in Central Europe and Asia, while a very close relative, *I. subelongatus*, is considered the most important xylophagous pest in Siberia and the north-eastern part of European Russia. In Denmark, *I. cembrae* has been spreading since its discovery in 1995, and the damage associated with this insect is serious (Harding *et al.* 1996).

In addition to preventive measures that aim to stop the arrival of invasive species, insight into the spatiotemporal dynamics of introduced species is a necessary part of understanding how new species spread and get established in new habitats. Unfortunately, the detection of invasive species usually lags several years behind their arrival (Shigesada and Kawasaki 1994). Successful efforts in forest management to prevent establishment of new species are dependant on a well-developed ecological theory for these systems. Several novel aspects of ecological theory of relevance to management will be explored in the present project. First, while much of ecological theory on population cycles and outbreaks invokes predation as the driving force (Begon *et al.* 1986, Krebs 1978), the population dynamics of bark beetles are resource-driven (Økland and Berryman 2004, Økland and Bjørnstad 2006). Second, the resource dynamics of bark beetles are highly stochastic. Most studies of resource exploitation and competition are based on mean-field theory, often leading to simple dynamics. In contrast, stochastic resource dynamics may lead to complex fluctuating patterns that are intermediate between cycles and chaos (Økland and Bjørnstad 2006). Third, the outbreak dynamics of bark beetles may be driven by a particular kind of resource dynamics that has received little attention in general ecological theory: when bark beetles reach a threshold density, they switch from their standard resources, such as dead or windfelled trees, to attacking and killing standing, apparently healthy trees (Berryman 1982, Økland and Berryman 2004). In this way, the organism essentially creates its own habitat and exploits an additional resource when the population reaches high densities. Finally, while we have begun to explore some of these issues in ongoing non-spatial analyses (Økland and Bjørnstad 2006), the spatial dynamics of these systems are completely unknown.

The spatial dimension has been shown to be fundamental to the dynamics in a number of systems, with important implications for management. Understanding the mechanisms behind spatio-temporal patterns of spread may be crucial to predict rates and patterns of spread, and assess how the management should act. Spatio-temporal dynamics ranging from frozen hotspots to waves, spirals and chaos can be generated in relatively simple systems (e.g. coupled map lattice models, Bjørnstad and Bascompte 2001). Spatially synchronous dynamics have been predicted by simulations and observed in several natural systems (Johnson *et al.* 2004, Bjørnstad *et al.* 2002), including *I. typographus* in Norway (Økland and Bjørnstad 2003). The niche overlap between bark beetles is known from several empirical studies (e.g. Nuorteva 1968). Theory predicts that spatial synchrony can be generated by three main mechanisms, dispersal, interactions and external forcing (Bjørnstad *et al.* 1999), and that the degree of synchrony depends on the strength of these forces. However, it is unclear how robust these generalizations are to non-linearity, threshold dynamics and spatial heterogeneity. This raises a number of questions that can be addressed in the bark beetle system. For instance, what patterns do such systems generate? How do the patterns, in particular outbreak patterns, change with respect to dispersal, degree of niche overlap and external forcing by climate? Are the fundamental aspects of the non-spatial single-species dynamics of the native bark beetle retained with the introduction of another species? What are the implications for management?

In a new project from 2006 to 2008, we will study the potential spread of introduced bark beetles, and evaluate their impacts on the spatiotemporal dynamics of the native spruce bark beetle. We will use an integrated approach that includes mathematical models and various aspects of ecology of bark beetles. Since experimental introductions of new species would be potentially very harmful, we will investigate these questions by a combination of mathematical models and time series analyses of population data. We will approximate an experimental situation using time series analysis and spatially explicit and realistic models based on existing time series of bark beetle observations and realistic landscape structures in a geographic information system (GIS). We will validate the model using patterns of spread of invasive bark beetles in other countries. Subgoals of the project are:

1. Develop a spatially explicit model for the native spruce bark beetle *Ips typographus*, and optimize it using time series analysis.
2. Expand the spatially explicit model of *I. typographus* to model the interaction dynamics of native and introduced bark beetles.
3. Explore the dynamics of the spatial interaction model.

The immediate outcome of our research will suggest possible changes in importation routines of wood and help forest managers to reduce the risk of spread and establishment of new bark beetles. For example, knowing the speed and pattern of spread will be important for deciding how quickly efforts should be put into action, and how large geographical areas should be involved in management plans to stop or slow down the spread of the invader. A deep understanding of the dynamics will also be useful to instruct forest industries how the risk of introductions and establishment can be minimized by changes of the importation routines. We will assess invasion risk and evaluate forest management strategies on the basis of mechanisms and observed patterns in the competition model, in particular with respect to timber import routines and scales of management. These recommendations will follow directly from the studies of spatial invasion dynamics described above. Examples of questions we will address are: how quickly does invasion propagate in space depending on species and environmental characteristics? What is the spatiotemporal distribution of outbreak patterns with the new species present? What are the relevant scales for control efforts? What are the most effective measures to prevent introduction and slow the spread?

References

Bakke A. 1989. The recent *Ips typographus* outbreak in Norway – experiences from a control program. Hol. Ecol. 12: 515-519.

Begon M., Harper J. L., Townsend C. R. 1986. Ecology: individuals, populations and communities. Blackwell Scientific Publications, Oxford.

Berryman A. A. 1982. Biological control, thresholds, and pest outbreaks. Environ. Entomol., 11: 544-549.

Bjørnstad O. N., Bascompte J. 2001. Synchrony and second order spatial correlation in host-parasitoid systems. Journal of Animal Ecology, 70: 924-933.

Bjørnstad O. N., Finkenstädt B., Grenfell B. T. 2002. Endemic and epidemic dynamics of measles. I. Estimating transmission rates and their scaling using a time series SIR model. Ecological Monographs 72: 185-202.

Bjørnstad O. N., Ims R., Lambin X. 1999. Spatial population dynamics: Causes and consequences of spatial synchrony in density fluctuations. Trends in Ecology and Evolution, 14: 427-431.

Bright D. E. 1976. The insects and arachnids of Canada, Part 2. Canadian Dep. Agric. Publ., 1576.

D'Antonio C., Kark S. 2002. Impacts and extent of biotic invasions in terrestrial ecosystems. Trends in Ecology and Evolution, 17:202-204.

Furniss R. L., Carolin V. M. 1977. Western forest insects. In. USDA For. Serv. Misc. Publ.

Heliövaara K., Peltonen M., Mannerkoski I., Siitonen J. 1998. Suomen Kaarnakuoriaiset (Coleoptera: Scolytidae). University of Helsinki, Department of Applied Zoology. Report 25.

Harding S., Pedersen A. F., Jørgensen M. L. 1996. Undersøgelser af barkbillen *Ips cembrae*. Skoven, 3:120-124.

Hoffard W. H. 1985. Southern pine beetle – a would-be manager of southern forests. [In:] Insect and disease conditions in the United States (eds R. C. Loomis , S. Tucker, T. H. Hofacker). USDA For. Serv. Gen. Tech. Rep., WO-46, pp 32-37.

Johnson D., Bjørnstad O. N., Liebhold A. 2004. Landscape geometry and traveling waves in the larch budmoth. Ecology Letters 7:967-974.

Krebs C. J. 1978. Ecology. The Experimental Analysis of Distribution and Abundance. Harper & Row Publishers, New York.

McGrath S., Farlow M. 2005. Attack of the alien invaders. National Geographic Magazine 207: 92-117.

McGregor M. D. 1985. Mountain pine beetle – the conflict between people and the beetle. [In:] Insect and disease conditions in the United States (eds R. C. Loomis, S. Tucker, T. H. Hofacker). USDA For. Serv. Gen. Tech. Rep. WO-46, pp. 16-23.

Nuorteva M. 1968. Über Mengenveränderungen der Borkenkäferfauna in einem südfinnischen Waldgebiet in der Zeit von 1953 bis 1964. Acta Entomologica Fennica 24: 7-50.

Økland B. 2002. Insekter, sopp og karplanter innført til Norge ved tømmerimport fra Russland og Baltikum. Aktuelt fra Skogforsk, 2/02:1-24.

Økland B., Bjørnstad O. N. 2003. Synchrony and geographical variation of the spruce bark beetle (*Ips typographus*) during a non-epidemic period. Population Ecology, 45: 213-219.

Økland B., Berryman A. 2004. Resource dynamic plays a key role in regional fluctuations of the spruce bark beetles *Ips typographus*. Agricultural and Forest Entomology, 6: 141-146.

Økland B., Kvamme T., Wollebæk G. 2005. Ny barkbilleart overvintrer ved tømmerlager. Skogeieren Skogeieren, 10-2005: 30-31.

Økland B., Bjørnstad O. 2006. A resource accumulation/depletion model of forest insect outbreaks. Ecology, 87(2): 283-290.

Pimentell D. (ed) 2002. Biological Invasions: economic and environmental cost of alien plant, animal and microbe species. CRC Press, Boca Raton, FL.

Shigesada N., Kawasaki K. 1994. Biological Invasions: Theory and Practice. Oxford Series in Ecology and Evolution.

Thunes K., Kvamme T., Økland B. 2003. Insekter innført ved tømmerimport. [In:] Insekter, sopp og karplanter innført til Norge ved tømmerimport fra Russland og Baltikum 2002 (ed. K. Thunes), 4/03: 15-20.

Werner R. A., Holsten E. H. 1997. Dispersal of the spruce beetle, *Dendroctonus rufipennis*, and the engraver beetle, Ips perturbatus in Alaska. [In:] USDA (ed. MYE! HMD). For. Serv. Res. Pap. PNW, pp 1-10.

Wood S. L. 1982. The bark and ambrosia beetles of North and Central America (*Coleoptera: Scolytidae*), a taxonomic monograph. Great Basin Nat. Mem., 6: 1-1359.

Wood C., Van Sickle G. A. 1992. Forest insect and disease conditions British Colombia and Yukon – 1992. Forestry Canada Pacific Forestry Centre.

INVADING INSECTS AND PATHOGENS IN SLOVAKIA FORESTS FOCUSING ON *DREYFUSIA NORDMANNIANAE* AS A REGULAR PEST IN MOUNTAIN AREAS

Milan Zúbrik, Andrej Kunca, Jozef Vakula, Roman Leontovyč,
Andrej Gubka

National Forest Centre, T.G. Masaryka 22, SK - 96092 Zvolen, Slovak Republic,
e-mail: zubrik@nlcsk.org

INTRODUCTION

Insect populations in ecosystems exist in a dynamic balance with the environment, members of the same species, other species, natural enemies etc. Each ecosystem is unique due to the occurrence of the particular mix of animals and plants. Interrelations of these organisms have formed mostly over a long time (several 10s up to several 100 thousand years) during which they have affected each other and adapted to each other. Every new organism that enters such system becomes a potential threat of disturbing the linkages and can cause avalanche negative reactions of autochthonous species. Invasions can be regarded as an abrupt and potentially mass penetration of a part of the population to a new territory, locality or habitat that can result in an outbreak in a new habitat. Namely an outbreak of invasive species may cause serious problems in forest ecosystems.

The invasion process is composed of three phases: arrival, establishment and spread. Major geographical barriers as oceans and mountain ranges have a great impact on the isolation of given populations. During periods of species range fluctuation (because of climate change, wind etc.) which result in crossing of borders, there may be rapid spreading to new areas. As a result of technological and sociological changes, people and their material goods are moved around the globe with ever-increasing efficiency and frequency. People have been during the last 200 years the most important factor increasing the rate of biological invasions (Liebhold *et al.* 1995; Kristin 1998).

The main types of invasions of in Slovakia based on occupied biotypes

Expansion across agricultural land. Colorado potato beetle *Leptinotarsa decemlineata* (in Slovakia since 1950), fall webworm *Hyphantria cunea* (since 1947), grape aphid *Phylloxera vitifolii* (since 1877). Expansion of the species *Quadraspidiotus* *perniciosus* from China across Northern America (1880) to Slovakia (1945) is well known. Such examples ilustrate that many economically significant species are spreading in regions of south Slovakia in the zone of Pannonian flora.

Expansion through old steppe. Fall webworm *Hyphantria cunea*, grape aphid *Phylloxera vitifoli,* Eastern pale-clouded yellow *Colias erate* and others. Most of these species are spreading mainly from the south.

Expansion of forest species – importation with plant material (wood, plants, fruits, leaves). Importation and subsequent increase in the abundance of Northern bark beetle *Ips duplicatus* in north-western Slovakia as well as spread of several defoliators like *Cameraria ohridella*, *Coleotechnites piceaella*, *Dreyfusia nordmannianae*, *Phyllonorycter robiniellus, Phyllonorycter issiki* etc.

Species introduced in the past

There is no doubt that many invading organisms become serious pests following their introductions in Slovakia. Several forest insect pests, which have been introduced to Slovakia in the past, are now so common that they are regarded as native in Slovakia.

Silver fir woolly aphid *Dreyfusia nordmannianae* Eckst was introduced to Slovakia from Asia at the end of the 19th century (Zúbrik 1998b). This small aphid is now one of the most important pests of young silver fir stands in the mountains of central Slovakia. Up to 500 ha are heavily infested annually and high levels of tree mortality in these stands is not an exception (Kunca *et al.* 2005).

Fall webworm moth *Hyphantria cunea* Drury was recorded for the first time in Budapest (Hungary) in 1940. The pest moved to Europe from Mexico and USA. As for Slovakia the pest was mentioned from the Slovak – Hungarian border (Komárno) between 1946–47. Since that time it has spread into most of the suitable areas in central and partially also in northern Slovakia (Jasic 1964). The pest is still common in Slovakia and causes problems in orchards and on fruit trees along roads (Kunca *et al.* 2005).

Cryphonectria parasitica was recorded for the first time in Duchonka – Prašice, Topoľčany district (Juhásová 1990) in 1976.

Recently introduced species

Among recently introduced species is horse chestnut leaf miner *Cameraria ohridella* Deschka & Dimic. It entered Slovakia in 1992–1994 and today almost all trees of horse chestnut *Aesculus hippocastanum* L. are infested by this species. Damage appears at the end of May and in July almost all foliage is damaged heavily.

An outbreak of Northern bark beetle *Ips duplicatus* was recordet in 1993 in northern Moravia. By 1997 foresters started to use pheromone traps for its monitoring and elimination in Slovakia, in southern part of Poland and Czech Republic. After that, the pest was found in northwestern part of Slovakia. Currently it represents a danger mainly for spruce stands at lower altitudes.

Phyllonorycter robiniellus that damages stands of robinia *Robinia pseudoacacia* L. originates from North America. This species was discovered for the first time in southwestern Hungary (1978–1979). In 1987 it was recorded for the first time in Slovakia and in 1989 in the Czech Republic. The pest mines the leaves of robinia trees.

Coleotechnites piceaella. By its way of life it is similar to several domestic species mining on spruce that are overwinter on the branches as the larval stage. Adult moths fly actively from June until August. Caterpillars feed on internal tissues of needles. To the end of the growth period of the tree larvae gnaw several needles and will winter in one of them. This species was found for the first time in Slovakia in Košice in 1990. It attacks spruce trees *Picea* spp. (mainly *Picea glauca*, *P. omorica, P. pungens)*.

Dothistroma septospora was first recorded in Slovakia during 1996 in Modrý Kameň, Veľký Krtíš district (Kunca, Foffová 2000). The first record was located close to the Hungarian border, and presumably the fungus was unintentionally introduced to Slovakia through the border by air at the beginning of 1990s. After that the area of its distribution has expended gradually around the entry point to the country. At present it can be found in forests with trees of the age 5–100 years in all regions of Slovakia.

In the year 2005 authors observed locally and in 2006 already in more places the occurrence of Eurasian species of miner *Phyllonorycter issikii.* Its habitat is similar to Northern bark beetle *Ips duplicatus* across Poland to the south and Central Europe. It is abundant for example around Levice and Hronský Beňadik. Information on the arrival has not yet been published.

In 2006 *Phytopthora alni* was found for the first time in Slovakia, in the area of the Low Tatra Mts. – Malužina (Kunca – personal communication). Information has not been published yet.

Species not registered yet but which are regarded as high risk

Asian race of gypsy moth L. It could be imported to Slovakia by transportation of wood and other material from Russia.

Asian longhorn beetle, *Anoplophora glabripennis* coming from China is a serious pest of poplar (*Populus* spp.), willow (*Salix* spp.), maple (*Acer* spp.), elm (*Ulmus* spp.) and some other tree species. It was recorded for the first time in the USA in 1996, where it became a serious danger in urban environments in New Jork and Chicago. It has been found breeding on trees in Austria and other countries of Europe.

Fire bugs such as *Leptoglossus occidentalis* and *Corythucha arcuata*, known from Italy, have not been found in Slovakia up to now. We have insufficient information on the occurrence of invasive Scolytidae, e.g. *Orthotomicus angulatus, Xyleborus germanus* and other known from Austria for example.

Phytopthora ramorum was detected in neighbouring countries such as Poland in 2000 (Orlikowski, Szkuta 2002), but not yet in the Czech Republic, Austria and

Hungary. This is a quarantine fungus, the cause of sudden oak death in California,which endangers Slovakia and soon might be detected in this region.

The aim of several experiments carried out recently was to evaluate the importance of *Dreyfusia nordmannianae* in forest and to verify some measures aimed at its control.

MATERIAL AND METHODS

Spatial distribution

The special governmental organization Forest Protection Service (FPS) of Slovakia is responsible for obtaining data about occurrence of forest damaging factors in Slovakia by the law 328/2005. All authors of the publication are members of the FPS and we used information collected by FPS.

The effect of Dreyfusia nordmannianae on the growth of forest

In the area of the Low Tatra Mts. we evaluated growth rate on 4 plots, each with an area of 2 hectares. In total we assessed 333 silver fir trees that were about 15-year old. Length of the terminal and lateral shoots were measured every year (in September).

Efficiency of ground and aerial treatments of Dreyfusia nordmannianae

In area of Harmanec 1 hect-are experimental plots in field experiments were established. The plots were treated in spring by ground treatment. We used the following preparations and concentrations: Hostaquic 50 EC 0.02%, Actelic 0.2%, Vaztak 10 EC 0.5%, Cyper extra 0.2%, Cyper extra 0.4%, Cyples 0.2%, Karate 2.5 EC 0.2%. Asse sment of efficacy was carried out after 40, 110 and 460 days. One series of the plots was treated by aerial treatment in autumn using a preparation Vaztak 10 EC at a cocentration of 6 l of the preparation in 100 l of water.

Bio-regulation spectrum of Dreyfusia nordmannianae

“Screening” for the presence of predators was carried out by branch tapping/oklepom on 4 research plots in the stands of spruce thickets in the area of Veľká Fatra Mts. The insects were tapped into a bin of 1×1 m. The bin was emptied always after 10 tappings. Ten collections were carried out during each day in each locality. Material was stored in 100 ml plastic bottles, sorted in a laboratory and partially stored for future identification (only larvae of Coccinellidae) on live food or they were identified immediately. The collections were performed on 28 May, 12 June, 14 June, 23 June, 27 June, 18 July and 24 August.

RESULTS

Spatial distribution

Species occurrence was situated in the central part of Slovakia. Figure 1 documents a certain degree of dependence of species occurrence on average annual temperatures in the studied area.

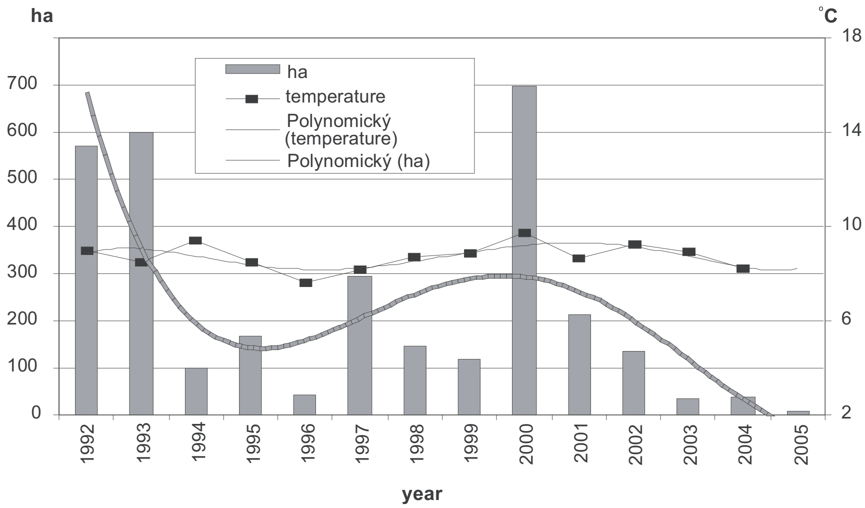


Figure 1. The occurrence of *Dreyfusia nordmannianae* since 1992 (source FPS). There are put into the graph the lines of trend and course of temperatures in the locality Banská Bystrica (data of Slovak Hydro meteorological Institute Bratislava)

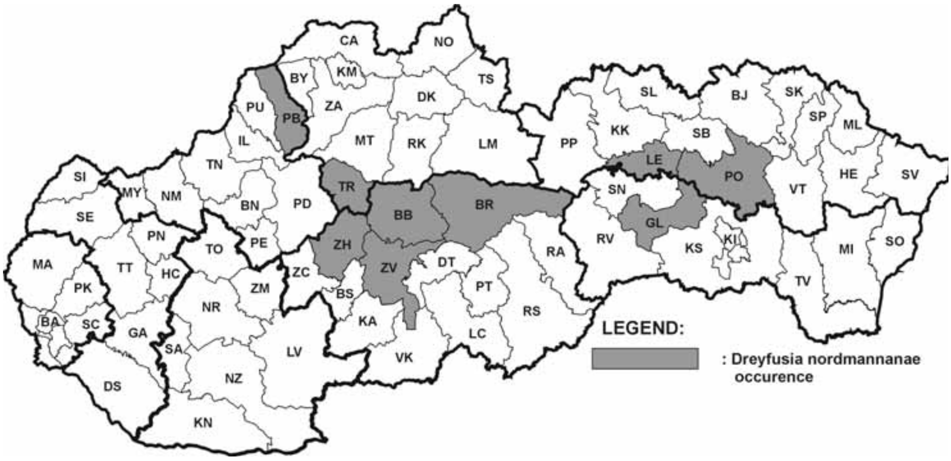


Figure 2. Spatial distribution of *Dreyfusia nordmannianae*

The effect of Dreyfusia nordmannianae on the growth of trees

The adelgid *Dreyfusia nordmannianae* affected markedly the increment of attacked trees. Table 1 clearly documents it.

Table 1. The effect of *D. nordmannianae* infestation on the length of the terminal and lateral shoots ** Significance on the level $P<0.05$

Tree infection in % (whole tree)	Number of trees	Terminal shoot length (cm)	Side-shoot length (cm)
0	190	28.5 ± 19.7	14.8 ± 8.4
1–20	58	20.3 ± 14.7	13.3 ± 6.3
21–40	26	17.8 ± 16.9	13.5 ± 6.5
41–60	21	10.9 ± 7.8	10.1 ± 2.8
61–80	26	3.3 ± 4.6	8.2 ± 3.2
81–100	12	3.4 ± 4.3	7.7 ± 2.8
Total/Statistics	333	**	**

Table 2. The ground treatment (spring application) as well as aerial treatment (autumn application) efficiency

Tree infection by the pest in % (whole tree)								
Insecticide	No. of trees	Day „0“	After the treatments					
			After 40 days		After 110 days		After 460 days	
		$X \pm S_x$	$X \pm S_x$	Change (%)	$X \pm S_x$	Change (%)	$X \pm S_x$	Change (%)
A	3×20	52.8 ± 16.2	35.3 ± 33.2	-33.1	36.7 ± 32.7	-30.4	31.8 ± 26.5	**- 39.7**
B	3×20	24.2 ± 17.3	6.3 ± 5.8	-75.8	9.5 ± 7.9	-60.7	26.3± 35.5	**8.6**
C	3×20	31.0 ± 13.0	7.0 ± 14.7	-77.4	7.0 ± 13.5	-77.4	7.0 ± 17.9	**- 77.4**
D	3×20	32.1 ± 17.6	6.7 ± 8.7	-79.1	6.0 ± 8.9	-81.3	9.7 ± 18.0	**- 69.7**
E	3×20	34.0 ± 19.6	3.7 ± 5.6	-89.1	3.0 ± 7.0	-91.1	3.5 ± 6.5	**- 89.7**
F	3×20	30.5 ± 16.3	10.4 ± 10.7	-65.4	11.0 ± 12.6	-63.9	17.5 ± 20.6	**- 42.6**
G	3×20	23.2 ± 18.7	3.4 ± 3.3	-85.3	3.7 ± 7.0	-84.0	4.5 ± 5.3	**- 80.6**
Aerial app.	3×20	38.2 ± 33.1	–	–	–	–	0.4 ± 2.33*	**- 98.9***
Control	3×20	30.7 ± 15.8	67.5 ± 26.7	+ 119.8	52.0 ± 19.6	+57.5	74.7 ± 28.9	**143.3**

Legend: change = difference from the before treatment status to the present status. A – Hostaquic 50 EC 0.02%, B – Actelic 0.2 %, C – Vaztak 10 EC 0.5%, D – Cyper extra 0.2%, E – Cyper extra 0.4%, F – Cyples 0.2%, G – Karate 2.5 EC 0.2 %, Aerial app. – Vaztak 10 EC 6 l /100 l, * after 330 days

Efficiency of ground and aerial treatment

Spring treatment improved substantially the health condition of fir stands, whereas on untreated control plots in the course of the second year almost 1.5 higher deterioration of the health condition followed. Cypermethrin used in this application ensured substantial improvement of the state of health in comparison with untreated controls. Aerial treatment appeared as the most efficient method of control.

Bio-regulation spectrum

The spectrum of predators was quite large. We recorded mainly larvae of Neuroptera and Syrphidae. The most frequent group was larvae as well as imago of Coccinellidae. Imago occurred less frequently than larvae. The most abundant species was the coccinellid *Brunus quadripustulatus* (79.4% of total number). It is the most abundant species of three species of the family *Brunus* occurring in Slovakia. We also found the species *Coccinela septempunctata* (17.6% of total number and *Coccinela bipunctata* (2.9% of total number).

CONCLUSION

Demonstrations of invasions of non-autochthonous organisms are frequent. We showed a significant effect of *Dreyfusia nordmannianae* on the stands in mountain regions of Slovakia and presented the list of species that occur in Slovakia and that can be expected in following years.

Literature

Jasic K. *et al.* 1964. Fall Webworm Moth (*Hyphantria cunea*). SAV Bratislava, 320 p.

Juhásová G. 1990. Nová choroba na gaštane jedlom [New disease of sweet chestnut]. Novosti vedy a techniky, 19, p. 12.

Kristin A. 1998. [Invasions of the animals in Slovakia and in Europe: the idea for discussion]. [In:] Invázie a invázne organizmy (ed. P. Eliáš). SNK SCOPE a SEKOS, Nitra, pp. 29 –40.

Kunca A., Brutovský D., Finďo S., Konôpka J., Leontovyč R., Longauerová V., Minďáš J., Novotný J., Pajtík J., Strmeň S., Vakula J., Varínsky J., Zúbrik M. 2005. The report about occurrence of the most injurious factors in the Slovak forests for the year 2004 and their forecast for the year 2005. FRI Zvolen, ISBN 80-8853-83-4, 90 p.

Kunca A., Foffová E. 2000. Ohrozenie porastov borovice čiernej fytokaranténnym patogénom *Dothistroma septospora* (Dorog) Morelet.[In:] Aktuálne problémy v ochrane lesa 2000 (ed. J. Varínsky). Zborník, Lesnícky výskumný ústav, Zvolen, p. 136-139.

Liebhold A. M., MacDonald W. L., Bergdahl D., Mastro V. C. 1995. Invasion by exotic forest pests: a threat to forest ecosystems. Forest Science Monograph 41.

Orlikowski L. B., Szkuta G. 2002. *Phytophthora ramorum* sp. nov. a new species in Poland recovered from *Rhododendron.* Phytopathologica Polonica 25, 69-79.

Zúbrik M. 1998. *Dreyfusia nordmannianae* and some aspects of its control. [In:] Lesy a lesníctvo pre tretie tisícročie (Zvolen 11–14. 10. 1998), Zborník referátov, pp.271-277.

BARK AND AMBROSIA BEETLE SPECIES IN WORLDWIDE TRADE

Miloš Knížek

Forestry and Game Management Research Institute, Jiloviste – Strnady,
Praha 5 – Zbraslav, Cz-156 04, Czech Repablic
e-mail: knizek@vulhm.cz

Wood and bark boring insect belongs among the most commonly transported insect inside the wooden products and wood packaging materials. Bark beetles are the most frequent group among these insects (Haack 2001; Brockerhoff *et al.* 2006). Serious forest pests were registered within the introduced bark beetles into new territories also, like *Scolytus multistriatus* (Marsham, 1802), a vector of the fungal pathogen causing Dutch elm disease (Webber 2000), and *Dendroctonus valens* LeConte, 1859, an invasive bark beetle that kills pines in China (Gao *et al.* 2005). No North American harmful bark beetle species was introduced into Europe till now (*X. germanus* and *G. materiarius* were established widespread, but are not harmful).

Species of the tribe Ipini, especially the genus *Ips,* containing the most destructive species, are also very frequent among these intercepted insects recorded during the phytoquarantine inspections in the ports of entry. Even though this fact, rather very few species of such genera were established outside the territory of their origin (Wood & Bright 1992; Bright & Skidmore 1997, 2002; author's identification). *Ips grandicollis* (Eichhoff, 1868)*, I. calligraphus* Germar, 1824 and *Orthotomicus angulatus* (Eichhoff, 1875) established in Australia, the latter one also in Fiji, *Orthotomicus caelatus* (Eichhoff, 1868) and *O. erosus* (Wollaston, 1857) were introduced into South Africa, *O. erosus* (Wollaston, 1857) and *O. laricis* (Fabricius, 1792) into Chile and *O. proximus* (Eichhoff, 1868) into Madagascar, and finally *Pityokteines curvidens* (Germar, 1824) was noticed as established in South Africa and Argentina. The occurrence of *P. curvidens* (Germar, 1824) in Japan and *P. spinidens* (Reitter, 1894) in Korea is questionable and some revision has to be done (Stark 1952).

Bark beetles were historically under the interest of entomologists and foresters mainly from very beginning, the first five species were described by Linné (1758) already. Scolytids belong to the group of insect with certain difficult determination. They spend most of their life time hidden in the host plant parts, they are generally uniformly one-colored, brown or black, of a small size and they share similar morphological characters. Series of local faunistic keys are available for determination of particular bark beetle species (e.g. Balachowsky 1949; Stark 1952; Nunberg 1954; Pfeffer 1955, 1995; Nobuchi 1971; Bright 1976; Wood 1982). All these papers deal with the local fauna only, not considering the whole range of diversity of all existing species in the genera. Due to the lack of comprehensive taxonomic keys, containing all species from the whole world ranged into particular genera, the taxonomy is remaining difficult. It causes serious problems with species identification of specimens collected during phytoquarantine inspections in the world wide trade. Noticed specimens could be easily wrongly determined or keyed out more times as very different species using several local faunal keys, when the place of origin of the specimens is not known properly, not mentioning the problems with different languages used in these particular sources.

The life of bark beetles – Scolytidae (in some literature they are ranged as subfamily of Curculionidae (Lawrence & Newton 1995) is closely related with their host plants. A big proportion of species belongs to the group of true bark beetles, which breed in and feed on the phloem (phloeophagous species) and many scolytids bore into the wood and feed primarily on symbiotic ambrosia fungi living in the tunnels (xylomycetophagous species). Some Scolytidae also develop in hard seeds and fruits (spermophagous species) and some in central pith of twigs and other small stems, or in the petioles of fallen leaves (myelophagous species) (Knížek & Beaver 2004). Generally, wood borers and bark beetles are frequently intercepted and can establish in other regions occasionally. These beetle groups contain many species that are significant forest pests and are of considerable risk to the biosecurity. Thanks to increasing international trade hundreds of species were detected by biosecurity, dozens species established, many introduced without detection. Any species from any part of the world could be received anywhere! Bark beetles, concealed and protected in the wood or under the bark, have better position to survive the travel in comparison with other insects. Most frequent cases of interceptions are in dunnage, casewood (crating) and sawn timber. Until now, the interception of new species is rather high, but their establishment is rare fortunately. Frequently intercepted species are likely to be established than rarely intercepted ones. In the same time the successful establishment is also abundance depending as well as behavioral strategy has a big role (*outbreeding* x *inbreeding*, xylomycetophagous development, and saproxylic tending (development in dying (dead) trees).

The behavior of newly intercepted species in new territory is unpredictable, they may become harmful or not, without dependence on their behavior in the place of their origin. In many cases their behavior is not known even in their original territory. There are many factors forming these relations, like successful reproduction, climate, suitable breeding material, natural enemies, inter-specific competition, host selection and many others. There are about 6000 species of bark beetles known worldwide, main centre of species diversity is tropical (and subtropical) region. About 600 species occur in North America, 900 species in Palaearct region, 250–350 species in Europe. In Czechia we have 111 species, from which approximately one third is of economic importance. Information about bark beetles are recorded in about 35–40 thousands papers! All together it is enormous amount of species and information to deal with. About 50 species are listed in the different quarantine lists of harmful organisms (quarantine lists, alert lists, national or regional lists, etc.). In some cases the whole family is considered, e.g. all Scolytidae (in EU and CR).

How we can expect new species in the territory? There are several passes to get a new species. It can be newly discovered species for science with its description, old cases (findings), when the species were known in the history and were suggested as extinct from particular area or in whole, by natural area movement caused by climatic changes or other means, or intended introduction, which is rather unlikely. The most probably pathway is accidental introduction by any of means mentioned previously (in wood and wood products mainly). We have several examples here in Europe of such introductions, for example *Xylosandrus germanus, Gnathotrichus materiarius, Dryocoetes himalayensis,* species of *Cryphalus, Hypothenemus*, etc.

Quite a few identification bugs were noticed during the revisions of the collections of intercepted bark beetle species, also in main and most harmful species (genera). Frequent misidentifications were found on generic as well as species level of the tribes Xyleborini, Dryocoetini, Ipini and Cryphalini mainly. Difficulties were noticed particularly in genera of *Orthotomicus, Ips, Phloeosinus, Polygraphus, Tomicus,* etc.

Information about introduced species can be obtained from different databases, lists, publications, etc. There is rather good record system in New Zealand, Australia, Canada, USA, but the situation with registering of new introductions in Europe, Asia, Africa and South America is not satisfactory. Databases are built thanks different reference diagnostic laboratories which are processing particular quarantine organism determination, by many forest research institutes and other institutions. Considerable information can be obtained also from different taxonomic catalogues (Wood & Bright 1992, Bright & Skidmore 1997, 2002) or local check-lists or monographies published by the taxonomists. But the amount of such information is primarily limited to species names and possibly specific locations only and mainly. What is regularly missing are the original citations of introductions, samples locations (collections, specimens) and introduction pathways. Many misleading are caused also by improperly use of the terms *intercepted* x *detected* x *introduced* x *established* throughout the literature.

For better understanding and easier determination the nomenclature for particular genera and species should be unified, as well as the keys containing all world wide known species in evaluated genera have to be developed. Advantages of fully illustrated computer based multi-entry diagnostic keys may lead to easier and faster determination of needed specimens to the species level, which is the fundamental act for decision making in pest risk assessment and could significantly minimize the financial expenses in forest protection or pest eradication programs. The keys have to be public available, on internet for example.

The needs are possible to define as necessity of early detection, correct identification, and study of behavior of particular species in places of their origin already. There are several tools which can lead to grant these needs in the same time. The uniform and public available international database system of detected and intercepted organisms, comprising maximum of targeted information as species name, pathway, original distribution, known economic importance etc., and proper identification [experts, identification tools (literature, interactive keys) and comparative material (collections)].

Except the recently established IUFRO WP 7.03.12 – Alien invasive species and international trade, there are also other working parties touching this topic. For example WP 7.03.10 – Methodology of forest insect and disease survey in central Europe, with the main mission “Improving forest insect and disease survey and advisory service in Central Europe and adjacent countries with similar natural conditions, forest stands and forest management”, has one of the specific objectives “Report of occurrence of new introduced species, harmful to trees and forests eco-systems”.

Literature

Balachowsky A. S. 1949. Faune de France 50. Coléoptères Scolytides. Paul Lechevalier, Paris, 320 pp.

Bright D. E. 1976. The insects and arachnids of Canada. Part 2: The bark beetles of Canada and Alaska, Coleoptera: Scolytidae. Canada Department of Agriculture, Research Branch, Biosystematics Research Institute, Publication 1576, 241 pp.

Bright D. E. & Skidmore R. E. 1997. A Catalog of Scolytidae and Platypodidae (Coleoptera), Supplement 1 (1990–1994). NRC Research Press, Ottawa, vii + 368 pp.

Bright, D. E. & Skidmore R. E. 2002. A Catalog of Scolytidae and Platypodidae (Coleoptera), Supplement 2 (1995–1999). NRC Research Press, Ottawa, viii + 523 pp.

Brockerhoff E. G., Bain J., Kimberley M., KníŽek M. 2006. Interception frequency of exotic bark and ambrosia beetles (Coleoptera: Scolytinae) and relationship with establishment in New Zealand and worldwide. Canadian Journal of Forest Research, 36: 1-10.

Gao B., Wen X., Guan H., Žiárek J., KníŽek M. 2005. Distribution and attack behaviour of the red turpentine beetle, *Dendroctonus valens,* recently introduced to China. Journal of Forest Science, 51: 155-160.

Haack R. A. 2001. Intercepted Scolytidae (Coleoptera) at U.S. ports of entry: 1985-2000. Integrated Pest Management Reviews, 6: 253-282.

KníŽek M., Beaver R. 2004. Taxonomy and systematics of bark and ambrosia beetles. [In:] Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis (eds. F. Lieutier , K. R. Daz, A. Battisti, J.-C. Grégoire, H.F. Evans). Kluwer Academic Publishers, Dordrecht, Boston, London, 41-54.

Lawrence J. F., Newton A. F. 1995. Families and subfamilies of Coleoptera (with selected genera, notes, references and data on family-group names). [In:] Biology, Phylogeny, and Classification of Coleoptera (eds J. Pakaluk , S. A. Slipinski). Papers Celebrating the 80th Birthday of Roy A. Crowson. Volume two. Muzeum I Instytut PAN, Warszawa, p. 559-1092.

Linné C. von. 1758. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata. Tomus I., reformata. Laurentii Salvii, Stockholm, iv + 824 pp.

Nobuchi A. 1971. Studies on Scolytidae IX (Coleoptera): Key to the subfamilies, tribes and genera of Japan. Bulletin of the Government Forest Experiment Station, 238: 149-164.

Nunberg M. 1954. Klucze do oznaczania owadów Polski, Cz. XIX Chrząszcze – Coleoptera, Zeszyt 99-100, Korniki – Scolytidae, Wyrynniki – Platypodidae. Panstwowe Wydawnictwo Naukowe, Warszawa, 106 pp.

Pfeffer A. 1955. Kùrovci – Scolytoidea. Fauna ÈSR, svazek 6. ÈSAV, Praha, 324 pp., 42 Tab.

Pfeffer A. 1995. Zentral- und westpaläarktische Borken- und Kernkäfer (Coleoptera: Scolytidae, Platypodidae). Pro Entomologia, Basel, 310 pp.

Stark V. N. 1952. Zhestkokrylye, Koroedy. Fauna SSSR. Akademia Nauk SSSR, Moskva, Leningrad, 463 pp.

Webber J. F. 2000. Insect vector behaviour and the evolution of Dutch elm disease. In Duun, C. P. (ed.). The Elms: breeding, conservation, and disease management. Kluwer, Boston, Mass, p.47-60.

Wood S. L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. Great Basin Naturalist Memoirs (6). Brigham Young University, Provo, Utah, 1359 pp.

Wood S. L., Bright D. E. 1992. A Catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: Taxonomic Index. Great Basin Naturalist Memoirs (13). Brigham Young University, Provo, Utah, 685 pp.

DISTRIBUTION OF TWO INVASIVE PESTS IN SLOVAKIA SINCE 1996

Jozef Vakula, Andrej Kunca, Milan Zúbrik, Roman Leontovyč,
Valéria Longauerová, Andrej Gubka

National Forest Centre, T.G. Masaryka 22, SK - 96092 Zvolen, Slovak Republic,
e-mail: vacula@nlcsk.org

INTRODUCTION

There are several pest agents of forest trees which have recently been introduced into Slovakia. In 1996 fungal pathogen *Dothistroma septospora* was identified in southern Slovakia. At the end of 1990s the pathogen was easily distributed to other parts of Slovakia. In 1996 the insect pest *Ips duplicatus* was caught for the first time in a pheromone trap in Zvolen. This species was later monitored all over Slovakia. Since 2000, we have been monitoring this pest just north-west part of Slovakia, however the area of its distribution is still growing. The poster describes these pest agents, their distribution since 1996 and their significance for forest ecosystems.

DOUBLE-SPINED BARK BEETLES *IPS DUPLICATUS* IN THE SLOVAK REPUBLIC

The double-spined bark beetle – *Ips duplicatus* (Fig. 1) was caught for the first time by Dr. Dušan Brutovský from Forest Research Institute in Zvolen in the year 1996 near Zvolen. In 1997, monitoring carried out over the whole territory of Slovakia discovered its appearance in central and north-west part of Slovakia.

Figure 1. The galleries of double-spined bark beetles *Ips duplicatus*

Figure 2. Catching of *Ips duplicatus* beetles in 2001

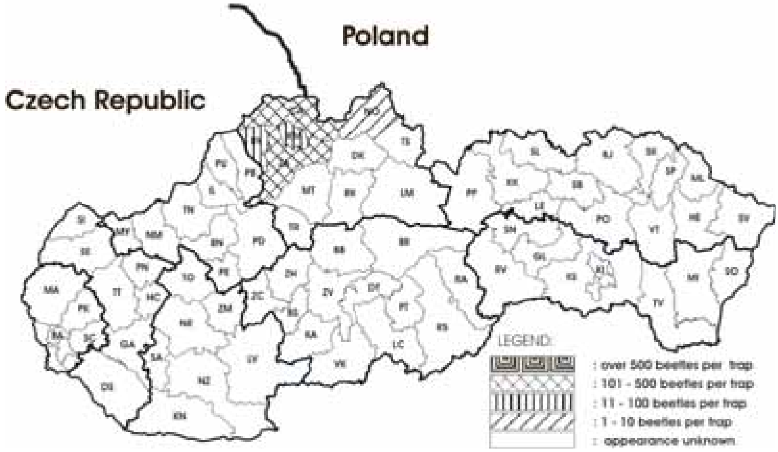


Figure 3. Catching of *Ips duplicatus* beetles in 2002

The population of *I. duplicatus* in the north-western part of Slovakia probably orginated from the frontier Polish region and also from the Czech Republic. For Central Slovakia it probably arrived on timber trade, primarily with rail-way transport.

Since the year 2001, periodical monitoring has been done in the chosen regions of Slovakia. The capture of this species in pheromone traps increases and its area spreads both southward and eastward (Fig. 2–6).

Retrograde conditions of spruce stands in recent years have had major effect on the expansion of the beetle. In the Slovak Republic it can be found at altitudes from 300 to 800 metres above sea level.

The attraction of *I. duplicatus* adults to pheromone traps baited with the pheromone ID-Ecolure in chosen districts of north-west Slovakia was demonstrated in 2001–2005 (Fig. 7).

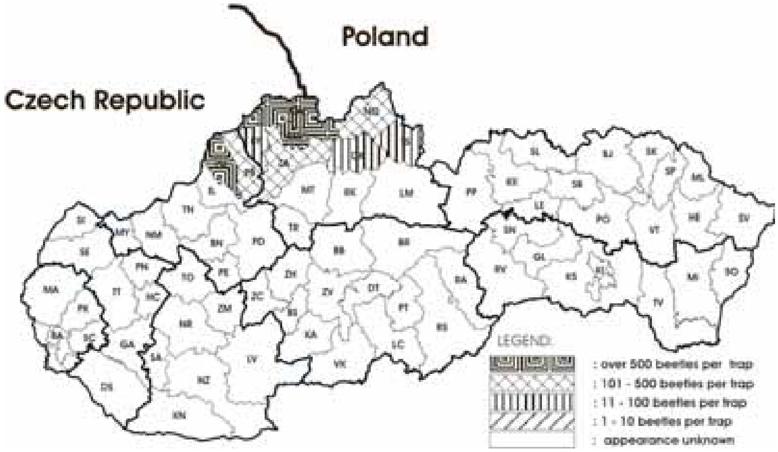


Figure 4. Catching of *Ips duplicatus* beetles in 2003

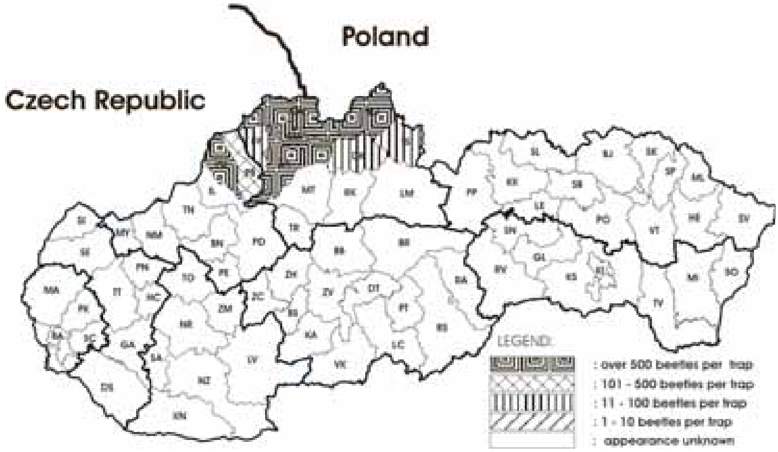


Figure 5. Catching of *Ips duplicatus* beetles in 2004

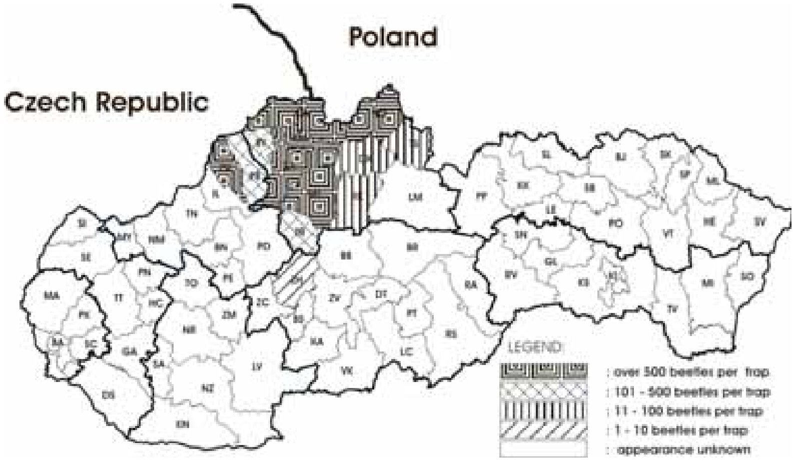


Figure 6. Catching of *Ips duplicatus* beetles in 2005

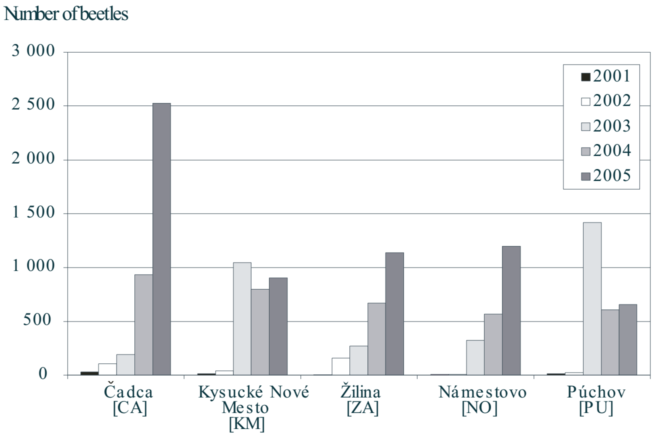


Figure 7. Development of double-spined bark beetle – *Ips duplicatus* catches to pheromone traps by pheromone ID-Ecolure in chosen districts of north-west Slovakia in 2001–2005

CONCLUSION

The area colonized by *I. duplicatus* is still growing. This species is one of the factors which account for large scale Norway spruce dieback in north-west Slovakia. It is time to change management of this bark beetle from monitoring to control by pheromone traps at least in north west Slovakia.

***Dothistroma septospora* (G. Doroguine) Morelet in the Slovak Republic**

First record in Slovakia: in 1996 in southern Slovakia close to Modrý Kameň, Veľký Krtíš district (Kunca, Foffová 2000).

Present status: The first entry was located close to the Hungarian border, presumably by accidental aerial introduction of the fungus into Slovakia over the border since the beginning of the 1990s. Over the following years the area of its distribution has been gradually growing around the entrance to the country (Fig. 8). At present, it can be found in forest aged from 5 through 100 years in all regions of Slovakia.

General comments: So far there is just little evidence of the occurrence of the fungus on seedlings and transplants in forest nurseries. This may be caused by intensive fungicidal control of needle cast diseases on Austrian pine as well as Scots pine. We have not found perfect stage *Mycosphaerella pini* Rostrup ap. Munk so far.

Hosts: *Pinus nigra* Arnold and *Pinus jeffreyi* have been the host species. These tree species are considered to be exotic to Slovakia. The disease is of concern mainly to growers of Christmas trees in plantations. There are some young plantations with extreme environmental conditions such as shallow soil, south facing slopes and dry climates where trees have died because of the severe *D. septospora* infection. At present we are testing potential control methods for the management of the disease.

As the disease is new to growers of Austrian pine, they need to know how to control the disease. We established two research plots in order to test efficacy of fungicidal spraying.

The crucial questions we stated were:

When to start spraying?

When to finish spraying?

Figure 8. Distribution of *Dothistroma septospora* in Slovakia since 1996

METHODS

The health condition of trees was evaluated prior to spraying in spring 2005 and for the second time in December 2005 in order to compare the efficacy of spraying with fungicidae. We evaluated the percentage of needles ranked according to "needles health degree" (Table 1). Each needle-year was classified separately.

Table1. Characteristics of needles health degree

Needles health degree	Statistical Weight of Health	Description
1	4	Needles green, without spots or chlorosis
2	3	Needles with spots of red, brown, yellow or other discolorations
3	2	Needles with brown top up to 1/3 of its length.
4	1	Needles with brown top up to 2/3 of its length.
5	0	Needles with brown top up to 3/3 of its length.

The index of health condition was calculated as multiplication of percentage (0–100), weight of needles age (1–3) and weight of needles health degree (0–4). The higher the index, the healthier the tree.

The significance of spraying we checked by Analysis of Variance.

RESULTS

The best results were obtained when spray application was carried out as early in the spring as possible (April). Results were still acceptable if spray applications commenced in June (Figure 9). There was no effect if control started in July or later.

Research plot in ŠS Šajdíkové Humence:

Pathogen: *Dothistroma septospora*
Locality: ŠS Šajdíkové Humence
Fungicide: Switch 62,5 WG
Concentration: 0,10%
Dosage of H_2O: 2,5 liters of suspension per 10 trees
Application: 2 x per month (at the beginning and at the end)
Period of control: April 2005 through October 2005
Age of trees: 6 years
No. of plots in the stand: 8
No. of trees in the plot: 10
Trees in total: 80

Question: *When to start spraying?*

	April		May		June		July		August		September		October	
	1.4.	15.4.	2.5.	16.5.	1.6.	15.6.	1.7.	15.7.	1.8.	15.8.	2.9.	16.9.	3.10.	
Plot 1	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	
Plot 2			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	
Plot 3					▨	▨	▨	▨	▨	▨	▨	▨	▨	
Plot 4							▨	▨	▨	▨	▨	▨	▨	
Plot 5									▨	▨	▨	▨	▨	
Plot 6											▨	▨	▨	
Plot 7													▨	
Plot 8 - control														

Research plot in Krnišov:

Pathogen: *Dothistroma septospora*
Locality: Krnišov
Fungicide: Switch 62,5 WG
Concentration: 0,10%
Dosage of H_2O: 2,5 liters of suspension per 10 trees
Application: 2 x per month (at the beginning and at the end)
Period of control: June 2005 through September 2005
Age of trees: 6 years
No. of plots in the stand: 5
No. of trees in the plot: 10
Trees in total: 50

Question: *When to finish spraying?*

	June	July	August	September
	2.6. and 16.6.	1.7. and 15.7.	1.8. and 15.8.	2.9. and 16.9.
Plot 1	▨	▨	▨	▨
Plot 2	▨	▨	▨	
Plot 3	▨	▨		
Plot 4	▨			
Plot 5 - control				

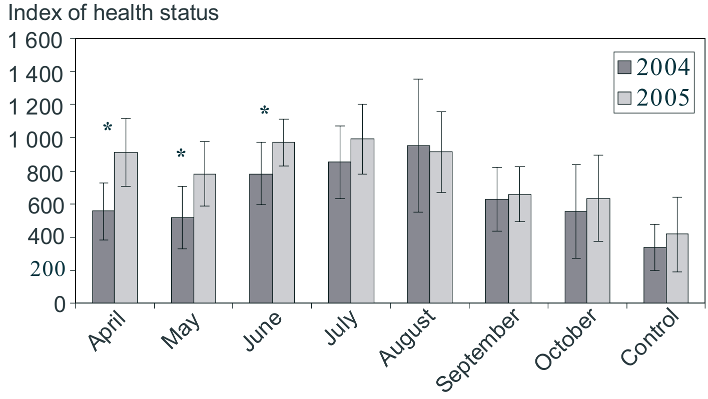


Figure 9. Chemical control of needles by Switch 62,5 WG, which started in different month but ended in October 2005 (Location: ŠS Šajdíkové Humence) (Asterix: P,05)

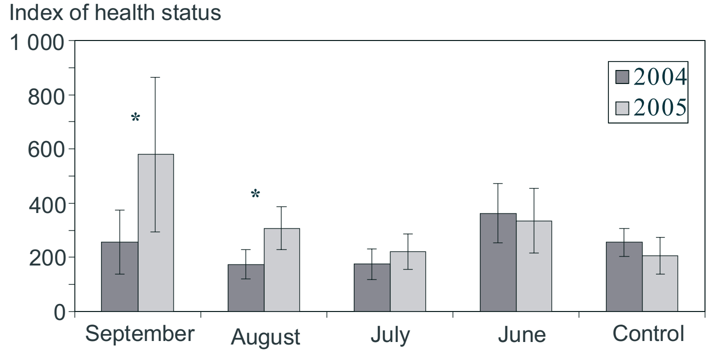


Figure 10. Chemical control of needles by Switch 62,5 WG, which started in June but ended in different month in 2005 (Location: Krnišov) (Asterix: P,05)

The best results were obtained when trees were treated for as long as possible during a year. It was necessary to apply fungicides at least to the end of August (Figure 10).

CONCLUSION

New diseases call for new information on many subjects. The disease caused by *D. septospora* is distributed throughout Slovakia and it is not practicable to apply eradication management. More important is proper control of plantations. Control of *D. septospora* infection of Austrian pine needles occurred under specific climatic conditions in Slovakia in 2005. We found that the pathogen can be controlled with fungicidal spraying, which should start as early in spring as possible (not later than in June) and should last at least to the end of August.

Literature

Kunca A., Brutovsky D., Findo S., Konopka J., Leontovyc R., Longauerová V., Mindas J., Novotny J., Pajtik J., Strmen S., Vakula J., Varinsky J., Zubrik M. 2005: The report about occurrence of the most injurious factors in the Slovak forests for the year 2004 and their forecast for the year 2005. FRI Zvolen, 90 p.

Kunca A., Foffová E., 2000: Ohrozenie porastov borovice čiernej fytokaranténnym patogénom *Dothistroma septospora* (Dorog.) Morelet. [In:] Aktuálne problémy v ochrane lesa 2000 (ed. J. Varínsky). Zborník, Lesnícky výskumný ústav, Zvolen, 136-139.

Kunca A., Longauerová V., 2006: Poznatky z ošetrovania borovice čiernej proti sypavkám. [In:] Aktuálne problémy v ochrane lesa 2006 (ed. A. Kunca), Zborník referátov z medzinárodného seminára,6-7.04.2006, Banská Štiavnica, 158-162.

FEW IMMIGRANT PHYTOPHAGOUS INSECTS ON WOODY PLANTS IN EUROPE: LEGACY OF THE EUROPEAN CRUCIBLE?

Henri Vanhanen[1], Timo Veteli[1], Sanna Sivonen[1], William Mattson[2],
Pekka Niemelä[1]

[1]Faculty of Forestry, University of Joensuu, POB 111, FI-80101 Joensuu, Finland;
e-mail: henri.vanhanen@joensuu.fi
[2]Forestry Sciences Laboratory, 5985 Highway K, Rhinelander, WI 54501, USA

INTRODUCTION

Phytophagous insects are continuously invading new biogeographic realms due to the ever increasing and substantial movement of commerce (Niemelä and Mattson 1996). Today, over 40% of the major North American invertebrate pests are of exotic origin, and more than half of its 2000 immigrant insect species are from the Western Palearctic (i.e. Europe, the Middle East and North Africa) (Sailer 1983; Wheeler and Henry 1992; Kim and McPheron 1993). Of the more than 400 immigrant species living on trees and shrubs in North America approximately 75% originate from Europe (Mattson 1994). The incredibly successful transfer of forest insects between Europe and North America is not surprising because of the high intensity of trade and human dispersal, and the substantial biogeographical similarity between these two continents (Rohrig and Ullrich 1991; Graham 1993).

Given these fundamental facts, one might hypothesize approximately equal insect immigration in both directions, or perhaps even higher NA to Europe immigration due to higher amounts of goods transported that direction during the past 500 years (Niemelä and Mattson 1996). An imbalance of bilateral commerce between Europe and North America has historically been most pronounced in agricultural and forest products. North America exports over fifteen thousand-fold more agricultural products such as cereals to Europe than vice versa (FAOstat 2005). The same biased pattern is true for forest products, especially the amount of untreated coniferous timber from North America. Yearly average ratios of NA exported to imported coniferous timber is 1,400,000/20,000 kg (EUROstat 2005).

Surprisingly, however, surveys of forest entomological literature show that about nine-fold more phytophagous forest insects from Europe have successfully invaded North America than vice versa (Sailer 1978; Mattson 1994; Niemelä and Mattson 1996). Based on several databases and published article, we have compared the invasive species in Europe with the data on European species established on woody plants in North America, and briefly discuss the reasons for the vast differences in invasive species numbers between the continents.

RESULTS AND DISCUSSION

To date, only 50 North American and 35 Asian woody phytophagous insect species have established permanent populations in Europe. In striking contrast, at least 310 tree/shrub feeding species have immigrated from Europe, and at least 77 species from Asia to North America (Mattson WJ, unpublished). Among the NA species introduced to Europe, Homoptera species clearly dominate. Second in abundance are Lepidoptera third are Coleoptera and fourth are the Hymenoptera (unpublished).

The same four taxonomic orders also dominate among European species that have successfully invaded North America. However, there are some interesting variations in their relative abundances. The percentage of Coleoptera among the European species that have invaded North America is two times higher (20.9 vs 10,0%) than among the American species that have invaded Europe. The reverse pattern is apparent for the Homoptera which are almost twice as abundant (44.0 vs 26.8%) among the invasives from North America to Europe than vice versa. Most of the host plant genera of NA and Asian invasive phytophagous insects are, in fact, their ancestral hosts imported into Europe. The same pattern in host plant use by immigrant phytophagous insects has been also recorded by Roques (2006) on coniferous plants.

There has been much scholarly speculation about the reasons for the biased insect trade between continents. The most often cited hypotheses for biased invasiveness are (1) differing species richness/abundance in donor and recipient population, (2) size and proportion of bilateral exchange, (3) ecological opportunities on arrival (hosts, etc.) and (4) ecological competitiveness of invaders. (Sailer 1983; Spence 1990; Vermeij 1991; Niemelä and Mattson 1996). The most likely explanations are the two final hypotheses which are discussed briefly below.

Discovering suitable host plants is undeniably the most crucial first step for a phytophagous immigrant to survive in the new habitat providet it can survive the new climatic conditions (Niemelä and Mattson 1996; Vermeij 1996). Host plants must be sufficiently abundant for their quick discovery and colonization by new immigrants (Niemelä and Mattson 1996). Plants are all important because they not only provide food and shelter for immigrants, but they also serve as platforms for rendezvous and mating. Europe generally has a much poorer flora than America because Pleistocence glaciations substantially diminished the richness of its pre-Pleistocene flora. At least 20 shared tree genera, and 58 related species became extinct in Europe but not in North America (Sauer 1989). Consequently, the reduced richness of woody species and genera in Europe makes it generally more difficult for the random, invading NA phytophagous species to establish successful populations. Obviously, polyphagous species, as well as those monophagous and oligophagous invading species that can readily adapt to a variety of confamilial host plants in the new environment will have a substantial advantage in finding suitable hosts.

After discovering a suitable host plant, the immigrant species will eventually face competitors, as well as a complex of new predators, parasites, and pathogens. The invading insect may fortuitously find an uncontested and broad ecological niche, but if not, it must simultaneously withstand all new natural enemies and out-compete alredy rivals (Vermeij 1996). European phytophagous insect communities have been hypothesized to be more resistant against new invaders due to their apparently higher species packing on plants (Niemelä and Mattson 1996). For further examination of these invasion ecology issues see Niemelä and Mattson 1996; Vermeij 1996; Carrol and Dingle 1996.

Because of their unique Pleistocene and Holocene evolutionary history, European species have been hypothesized to be especially adapted for invasions (Niemelä and Mattson 1996). According to this European crucible hypothesis, glaciations were much more effective in Europe in selecting for species that can cope with disturbance and fragmentation because its many west-to-east mountain chains, compared to North American north-to-south mountain chains, blocked species' retreats and repeatedly compartmentalized them into many disparate refugia in a staccato of ca. 12 glacial wave episodes. Since the last glacial maxima, rapid human population expansion has likewise contributed to extensive destruction and fragmentation of European forests and their inhabitants. In such refugia, related species often came into close contact and hybridized, forming asexual forms that were often polyploids and were typically much better colonizers than their parents (Niemelä and Mattson 1996; Stenberg and Lundmark 2004). For all of these reasons, many European species may be more likely to tolerate inbreeding, more able to cope with low population numbers, and rapid population expansion, and furthermore may be especially adept at tolerating a wide range of ecological conditions, i.e. having especially broad ecological amplitude, i.e. tri-trophic niches (Niemelä and Mattson 1996). The success of asexually reproducing species is most clearly evident in the dominance of Homoptera species as invaders, though the trait is also commonly found in Hymenoptera, and also in Coleoptera: Curculionidae and Scolytidae. European species seemingly dominate as invasive species compared to species from other continents, and this is probably due to the European crucible.

Acknowledgements. *We thank the Academy of Finland (Finnish Centre of Excellence Program 2000-2005, project no. 64308), Finnish Forest Industry and Maj and Tor Nessling foundation for financially supporting this study. We also thank John Derome for improving the language of the paper and Seppo Neuvonen for helpful comments.*

References

Graham A. 1993. History of vegetation: Cretaceous (Maastrichtian)-Tertiary. [In:] Flora of North America Committee (eds) Flora of North America north of Mexico. Oxford University Press, New York, Vol. I: 57-70.

EUROstat, 2005. EUROstat data. European Union. Retrieved from http://epp.eurostat.cec.eu.int/portal/ page?_pageid=1090,1&_dad=portal&_schema=PORTAL on 16 June 2005.

FAOstat, 2005. FAOstat data. Food and Agriculture Organization of the United Nations. Retrieved from http://faostat.fao.org/faostat/collections?version=ext&hasbulk=0&subset=forestry) on 10 June 2005.

Kim K. C., McPheron B. A. (eds), 1993. Evolution of Insect Pests: Patterns of Variation. John Wiley, New York, 479 pp.

Mattson W. J., Niemelä P., Millers I., Inguanzo Y. 1994. Immigrant phytophagous insects on woody plants in the United States and Canada: An annotated list. General Technical Report NC-169. St Paul (MN), US Department of Agriculture.

Niemelä P., Mattson W. J. 1996. Invasion of North American forests by European phytophagous insects: legacy of the European crucible? BioScience, 46: 741-753.

Rohrig E., Ulrich E. (eds), 1991. Ecosystems of the world. Temperate deciduous forests. Elsevier, New York. Vol. 7.

Roques A., Auger-Rozenberg and M-A, Boivin S. 2006. A lack of native congeners may limit colonization of introduced conifers by indigenous insects in Europe. Can. Gentlemen. Forest Research, 36/2: 299-313.

Sailer R. I. 1978. Our Immigrant Insect Fauna. ESA Bulletin 24(1).

Sailer R. I. 1983. History of insect introductions. [In:] Exotic plant pests and North American agriculture (eds C. L. Wilson, C. L. Graham), Academic Press, New York, pp15-38.

Sauer J. D. 1988. Plant migration: the dynamics of geographic patterning in seed plant species. University of California Pres, Berkley, California. 282p.

Spence J. R. 1990. Success of European carabid species in Western Canada: preadaptation for synanthropy? [In:] The role of ground beetles in ecological and environmental studies (ed. N. E. Stork), Intercept, Andover, Hampshire: 129-141.

Stenberg P., Lundmark M. 2004. Distribution, mechanisms, and evolutionary significance of clonality and polyploidy in weevils. Agriculture and Forest Entomology, 6: 259-266.

Vermeij G. J. 1991. Anatomy of an invasion: the trans-Arctic interchange. Paleobiology, 17(3): 281-307.

Vermeij G. J. 1996. An Agenda for Invasion Biology. Biological conservation, 78: 3-9.

Wheeler A. G., Henry T. J. 1992. A synthesis of the Holarctic Miridae (Heteroptera): distribution, biology and origin with emphasis on North America. Latham (MD): The Thomas Say Foundation, Entomological Society of America.

ECOLOGICAL IMPACT OF INVASIVE INSECTS IN FOREST ECOSYTEMS

Marc Kenis, Christelle Péré

CABI Europe-Switzerland, 1 Rue des Grillons, 2800 Delémont, Switzerland
e-mail: m.kenis@cabi.org; c.pere@cabi.org

INTRODUCTION

Non-indigenous insects are particularly harmful to the economy, as pests in agriculture, horticulture, stored products or forestry (Pimentel 2002). In addition, many invasive insects are known to threaten native biodiversity and ecosystems. The ecological impact of non-indigenous insects on the environment can be observed at genetic, individual, population, community and ecosystem level. It may occur at different spatial scales, from microhabitat to landscape, and through various mechanisms, such as predation, competition, herbivory, hybridization, etc.

In this paper, we present a review of the most outstanding cases of ecological impacts caused by alien insects in forest ecosystems, and the mechanisms underlying these impacts. Most cases cited in this review come from North America or Australasia. In other regions (e.g. Europe, South America, Asia, Africa), the ecological impact of invasive insects has been much less studied, partly because insect invasions have been less critical for the environment in these regions than in North America, Australasia and most oceanic islands. For example, in a literature study on 341 alien insects established in Switzerland and Austria, Kenis *et al.* (submitted) found no evidence of ecological impact.

GENETIC IMPACT

Hybridization between invasive and native insect species or sub-species has rarely been documented, with the notable exception of honey bee and bumble bee sub-species (e.g. Goulson 2003). There is no case of reported genetic impact on native species in forest ecosystems. The fact that hybridization is a common phenomenon between alien and native species or sub-species in other taxonomic groups such as mammals (Long 2003), amphibians (Vorburger and Reyer 2003) or plants (Vilà *et al.* 2000) suggests that the lack of cases in insects does not reflect a true situation but is rather due to a lack of knowledge and investigations.

IMPACT ON POPULATIONS AND COMMUNITIES OF NATIVE SPECIES

Herbivory on native flora

Most reported cases of impact of invasive insects on native biodiversity concern observations of alien herbivores affecting native plants. However, many of these are anecdotal observations of an alien insect damaging or killing individual plants. Few studies provide data on the long-term, quantitative impact on alien herbivores on plant populations and communities. The best documented and most spectacular cases of herbivores affecting native forest plants are several Eurasian forest pests threatening native trees and their ecosystems in North America. For example the balsam woolly adelgid, *Adelges piceae*, and the hemlock woolly adelgid, *Adelges tsugae*, are threatening unique forest ecosystems in eastern North America by killing hemlock (*Tsuga* spp.) and fir (*Abies* spp.), which are gradually replaced by other tree species (Orwig and Foster 1998; Smith and Nicholas 2000). Similarly, since its introduction into New England in the 19th century, the European gypsy moth, *Lymantria dispar*, has had a serious impact on eastern North American oak forests (Allen and Bowersox 1989).

Rare, endemic trees on oceanic islands may be particularly threatened by the invasion of non-indigenous forest pests. For example, in St. Helena, the scale insect *Orthezia insignis*, nearly drove the endemic gumwood *Commidendrum robustum* to extinction when a successful biological control programme was implemented (Fowler, 2004). In Hawaii, the ambrosia beetle *Xylosandrus compactus* is attacking several rare and threatened endemic trees (Ziegler 2002).

Predation on, and competition with native fauna

Invasive insects may displace native animal species through direct predation and/or competition for food and space. The multicoloured Asian ladybeetle, *Harmonia axyridis*, and the European seven spotted lady beetle, *Coccinella septempunctata*, are known to displace native ladybirds in agricultural ecosystems in North America (Elliott *et al.* 1996; Koch 2003). The mechanisms underlying this displacement are not clearly understood, but are probably a combination of direct predation on native ladybird eggs and larvae and competition for food.

Among predatory insects, ants are undoubtedly those for which ecological impacts have been most documented. Several invasive ant species seriously affect the native fauna of various ecosystems, including forests. A good example is the crazy ant, *Anoplolepis gracilipes*, which preys on the red land crab on Christmas Island, decimating its populations and causing important changes in this unique forest ecosystem (O'Dowd *et al.* 2003). The ant reduces crab populations, resulting in slower litter breakdown, releases of seedling recruitment and the increase of tree and species richness. In addition, a new association between the ant and scale insects leads to tree dieback and species replacement.

Parasitism on native fauna

Invasive insects can affect the native fauna through parasitism on native insects or vertebrates. Parasitoids are usually considered as beneficial insects and numbers of species have been released in new environments worldwide for biological control purpose. Although many of these introductions are considered positive, in a few cases the parasitoids have become a threat to non-target insects. An example in forest ecosystems is the tachinid fly, *Compsilura concinnata*, introduced from Europe in North America at the beginning of the 20th century to control the gypsy moth. *C. concinnata* is now suspected of having a negative impact on populations of endangered native saturniid moths (Boettner *et al.* 2000). Invasive ectoparasites of vertebrates may also affect native species, including in forest ecosystems. For example, a chewing louse, *Damalinia* sp. causes hair-loss syndrome in blacktail deer in western North America (Bildfell *et al.* 2004).

Vectors of diseases

Invasive insects may affect the native flora and fauna by transmitting or facilitating diseases. The European bark beetle, *Scolytus multistriatus*, has become the main vector of the Dutch elm disease, *Ophiostoma ulmi* and *O. novi-ulmi*, in North America (Brasier 2000). The European beech scale, *Cryptococcus fagisuga*, is associated with the fungus *Nectria coccinea* var. *faginata* to cause beech bark disease in North America (Houston 1994). Native animals may also be affected, such as several endemic birds in Hawaii, which are decimated by avian malaria transmitted mainly by alien mosquitoes (LaPointe *et al.* 2005).

Indirect competition through shared of natural enemies

Other indirect impact mechanisms may occur between alien and native species. In particular, an alien insect may compete with a native insect through the share of natural enemies (Holt & Lawton 1993). There is no published example of this mechanism in forest ecosystems but there are ongoing studies on the impact of the invasive horse-chestnut leaf miner, *Cameraria ohridella*, on native leaf miners in Europe, through shared parasitoids (C. Péré and M. Kenis, unpublished).

IMPACT ON ECOSYSTEMS

Invasive insects, by affecting native species populations and communities, may also cause important changes in ecosystem processes and services. Most of the best illustrated cases of ecosystem alteration caused by invasive insects have been studied in forest ecosystems. For example, outbreaks of the gypsy moth, *Lymantria dispar*, in eastern North America have resulted in dramatic changes in the functioning of oak forests. The following consequences of defoliation have been cited: decrease of transpiration, tree growth and seed production; increase of tree mortality, light penetration and water drainage; alteration of tree species composition and, consequently, faunistic composition; alteration of carbon allocation and nitrogen cycling, which, among others, results in acidification of stream waters (Lovett *et al.* 2003). The mortality of hemlock trees in North America, caused by the hemlock woolly adelgid, *Adelges tsugae*, has im*et al.* 1999).

CONCLUSIONS

The ecological impact of invasive insects has been poorly studied compared to, e.g., plants or vertebrates. Most observations of impact are anecdotal. Good quantitative studies are rather rare, especially those that clearly identify the mechanisms underlying these impacts.

However, several of the most remarkable studies on the ecological impact of invasive insects have been carried out in forest ecosystems. Among the best illustrated cases are the direct impacts of herbivores on native tree populations and of predators and parasitoids of the native fauna. In contrast, indirect impact mechanisms are poorly studied and understood. There are few good studies on ecosystem impacts, and these are limited to some forest herbivores and ants. Genetic introgression has been very rarely observed, but this is probably at least partly due to a lack of knowledge. It is interesting to note that there is a strong bias towards impacts on trees compared to shrubs or herbaceous plants, probably because hazards on trees are more obvious and because of the importance of trees as key species in forest ecosystems.

Acknowledgements. *This study has been funded by the EU FWP6 project ALARM (GOCE-CT-2003-506675) and the Loterie Romande.*

References

Allen D., Bowersox T. W. 1989: Regeneration in oak stands following gypsy moth defoliations. [In:] Proc. 7th Central Hardwood Conference, General Technical Report (eds G. Rink, C. A. Budelsky). NC-132. United States Department of Agriculture Forest Service, North Central Experiment Station, 67-73.

Bildfell R. J., Mertins J. W., Mortensen J. A., Cottam D. F. 2004: Hair-loss syndrome in black-tailed deer of the Pacific Northwest. Journal of Wildlife Disease, 40: 670-681.

Boettner G. H., Elkinton J. S., Boettner C. J. 2000: Effects of a biological control introduction on three non-target native species of saturniid moth. Conservation Biology, 14: 1798-1806

Brasier C. M. 2000: Intercontinental Spread and Continuing Evolution of the Dutch Elm Research Pathogens. Kluwer Academic Publishers, Boston, USA, 61-72.

Elliott N., Kieckhefer R., Kauffman W. 1996: Effects of an invading coccinellid on native coccinellids in an agricultural landscape. Oecologia, 105: 537-544.

Fowler S. V. 2004: Biological control of an exotic scale. *Orthezia insignis* Browne (Homoptera: Ortheziidae), saves the endemic gumwood tree *Commidendrum robustum* (Roxb.) DC. (Asteraceae) on the Island of St. Helena. Biological Control, 29: 367-374.

Goulson D. 2003: Effects of introduced bees on native ecosystems. Ann. Rev. Ecol. Syst., 34: 1-26.

Holt R. D., Lawton J. H. 1993: Apparent competition and enemy-free space in insect-host parasitoid relationship. American Naturalist, 142: 623-645.

Houston D. R. 1994: Major new tree disease epidemics: Beach bark disease. Annual Review of Phytopathology, 32: 75-87.

Jenkins J. C., Aber J. D., Canham C. D. 1999: Hemlock woolly adelgid impacts on community structure and N cycling rates in eastern hemlock forests. Canadian Journal of Forest Research, 29: 630-645.

Kenis M., Rabitsch W., Auger-Rozenberg M. A., Roques A. Submitted: How can alien species inventories and interception data help us prevent insect invasions?

Koch R. L. 2003: The Multicolored Asian Lady Beetle, Harmonia Axyridis: A Review of Its Biology, Uses in Biological Control, and Non-target Impacts. 16pp. Journal of Insect Science, 3:32, Available online: insectscience.org/3.32.

LaPointe D. A., Goff M. L., Atkinson C. T. 2005: Comparative susceptibility of introduced forest-dwelling mosquitoes in Hawaii to avian malaria, *Plasmodium relictum*. Journal of Parasitology, 91: 843-849.

Long J. L. 2003: Introduced Mammals of the World. Their History, Distribution and Influence. CABI, Wallingford, UK, 589 pp.

Lovett G. M., Christenson L. M., Groffman P. M, Jones C. G., Hart J. E., Mitchell M. J. 2002: Insect defoliating and nitrogen cyclingin forests. Bioscience, 52: 335-341.

O'Dowd D. J., Green P. T., Lake P.S. 2003: Invasion "meltdown" on an oceanic island. Ecology Letters, 6: 812-817.

Orwig D. A., Foster D. R. 1998: Forest response to the introduced hemlock woolly adelgid in southern New England, USA. Journal of the Torrey Botanical Garden Society, 125: 60-73.

Pimentel D. (ed.) 2002: Biological Invasions. Economics and Environmental Costs of Alien Plant, Animal, and Microbe Species. CRC Press, Boca Ratton, USA.

Smith G. F., Nicholas N. S. 2000: Size- and age-class distributions of Fraser fir following balsam woolly adelgid infestation. Canadian Journal of Forest Research, 30: 948-957.

Vilà M., Weber E., D'Antonio C. 2000: Conservation implications of invasion by plant hybridization. Biological Invasions, 2: 207–217.

Vorburger C., Reyer H.-U. 2003: A genetic mechanism of species replacement in European waterfrogs? Conservation Genetics, 4: 141-155.

Ziegler M. 2002: Uhiuhi. Environment Hawai'i, 12: 1-2.

THE NEGATIVE INFLUENCE OF THE SO-CALLED ”INJECTION” MEASURE ON THREATENED HORSE CHESTNUT TREES

Tomasz Oszako[1], Marek Siewniak[2], Margarita Siewniak[3]

[1]Research Institute, Department of Forest, Phytopathology, Sekoçin Las, Raszyn, Poland;
e-mail: T.Oszako@ibles.waw.pl
[2]Institute of Landscape Architecture, Kraków Technical University
[3]M. Eng., Plant Breeding Station, Pawłowice

INTRODUCTION

For several decades now, horse chestnut trees (*Aesculus hippocastanum*) have been afflicted by *Guignardia* leaf blotch, as caused by the fungus *Guignardia aesculi* which was brought to Europe from America in the 1930s. In the 1970s, 80s and 90s it was possible to observe horse chestnut trees in city streets with brown-spotted leaves being lost as early as in June (Moreth *et al.* 2001). Characteristically, there was a second growth of small leaves in July–August, while inflorescences tended to be small and under-developed. Since 1998, horse chestnuts have also been subject to attack by the horse-chestnut leaf-miner (*Cameraria ohridella*). These two biotic factors, taken together with increased salinity and other burdens imposed in city streets (like soil loss and oxygen starvation of roots) have been sufficient to exert a synergistic impact on horse chestnuts for several decades now. Nevertheless, it was only when the mass outbreaks of the leaf-miner reached the attention of the media that public demands for this favourite tree species to be saved began to appear. The horse-chestnut leaf-miner has today achieved the status of important pest, this being linked with the capacity of mass outbreaks to spread very rapidly, as well as with the importance of the horse chestnut as a familiar tree widely cultivated in parks and gardens, in cities, along streets and avenues and even in the open landscape and in forests (Plate 1).

THE ORIGIN OF THE INSECT

The horse-chestnut leaf-miner was first described by Deschke and Dimica in 1986, from the area of Macedonia near Lake Ohrid, where it appeared *en masse* in 1984.

However, there is much to suggest that the species is not of Balkan origin. It is, for example, indicative that there are no known antagonists for it in Europe (at least according to the 10 years of Austrian observation by Tomicek (1998, 2001). The genus *Cameraria* is associated with trees of the genus *Aesculus*, and could therefore have originated in Asia, North America or southern Europe. However, it seems that *C.*

Plate 1. External symptoms of feeding on leaves by the horse-chestnut leaf-miner

ohridella's closest relative is the leaf-miner *C. aesculisella*, which lives on *A. glabra* and *A. flava* in the United States.

The first Central European records of the species came from the Linz and Vienna areas of Austria in 1989. However, in the course of the subsequent several to 10+ years, the dramatic spread of severe outbreaks of the leaf-miner encouraged gardeners into something of a panic approaching psychotic dimensions. This reflects the dramatic nature of the attack phenomenon, the lack of resistance in the natural environment, and the apparent helplessness of people to intervene. The shock is further amplified by what is thus far a lack of any significant known parasites or predators of the pest species.

To be added to this are the several decades of observations concerning attacks on horse chestnuts by the blight or leaf blotch that the ascomycete fungus *Guignarida aesculi* is capable of giving rise to. This fungus was imported into Europe from the USA in the 1920s or 30s, and has been causing ever more widespread weakening, dieback and death in horse chestnuts. As this phenomenon was never as dramatic as the debilitation caused by the leaf-miner, its seriousness tended to be played down and to go unobserved, notwithstanding a number of warnings issued.

THE SPREAD OF THE PEST

The rapid spread of what is a small moth may have been facilitated by Europe's heavy road traffic, alongside both passive and active migrations on the part of specimens. Doubtless passive spread was favoured by the fringed wings of the moth. First records of the species were:

1984 – Macedonia (Lake Ohrid),
1989 – Zagreb (Croatia), Albania, Austria – the Linz area,
1992 – Italy, Austria,
1993 – Hungary, Slovakia, Czech Republic, Bavaria
1998 – Germany, Denmark, The Netherlands, France, Bulgaria, Poland – Upper and Lower Silesia,
2000 – Poland (Cieszyn, Warsaw).

THE BIOLOGY OF THE PEST

The moth emerges from pupae overwintering in leaf litter at the end of April, or in the first third of May should the winter have been a hard one. The moth is about 5 mm long, with a wingspan of c. 7mm. It is a metallic ochre colour and has three white stripes or bars. Mass emergences from the silky cocoons in leaf litter may occur. There is swarming in the lower parts of tree crowns in the first half of May. The appearance of a large number of moths at trunk height is a very distinct signal.

Eggs

The female lays c. 20 (40–60) transparent whitish eggs that are not visible to the naked eye. These are left on the upper side of leaves, mainly on side veins.

Larvae

The insect’s development may pass through five larval stadia. Larvae emerge from eggs some 2–3 weeks after laying. They chew through the leaf cuticle into the softer tissue of the pallisade layer, feeding there for some 20–30 days.

The mines the larvae produce enlarge gradually from small, light-coloured circular ones to wide dark patchy ones up to 4.5 cm long. Ever greater amounts of faeces accumulate within, and the pupa is ultimately located here.

The degree to which a tree has been taken over can be determined in terms of the numbers of mines on leaves.

Pupa

First-generation (spring) pupae in their casing appear around the middle of June. The second-generation imago emerges 2–3 weeks later, and develops in a similar way to the first-generation form. It is usual for the species to pass through two generations in Poland too, the large numbers of second- (summer-) generation pupae residing within silky cocoons that facilitate overwintering. There may even be a third (autumn) generation in southern Europe. Pupae appear capable of surviving temperatures as low as -23^0C, though extra frosty winters do result in decimation of numbers of living pupae, unless these gain protection in warm litter layers.

Mines made by the horse-chestnut leaf-miner have also been noted on sycamore (as well as on other members of the *Hippocastanaceae*, especially the Japanese horse chestnut *Aesculus turbinata*). Nevertheless, while these other species are inhabited by miners, the development of the pests would seem to go uncompleted in these cases.

ENVIRONMENTAL RESISTANCE AND COMPETITION

The level of environmental resistance remains exceptionally low and of no practical significance, especially in street locations. It may nevertheless be developing gradually.

The existence of a certain group of antagonistic predatory insects is indicated where the tree grows in parks and forests.

It is usual for food in the form of leaves to be inadequate where second- and third-generation leaf-miners are present in large-scale outbreaks. It is this lack of food which is currently regarded as the most important “natural” factor limiting populations of the pest.

PROBLEMS ASSOCIATED WITH THE PROTECTION OF TREES

Various measures have thus far been taken in an attempt to combat the pest. The appearance of leaf-miners on trees in parks may point to some kind of ”initial” stage of the problem, quite in contrast to the crisis situation now affecting urban horse chestnuts. In the latter case, an urgent need to alleviate what are in general burdensome environmental conditions is indicated, since there is a lack of resistance to the new pest and legal restrictions on the use of chemical plant protection agents in towns and cities.

The spraying of tree crowns in an effort to combat larvae has involved chitin-biosynthesis inhibitors. These compounds are also known to kill the eggs of certain insect species. With a view to populations of the horse-chestnut leaf-miner being limited, the trunks of lower branches of trees can be sprayed with emulsions of contact insecticides. However, regulations on the protection of the urban environment mean that such measures are of limited use. As a result, investigations concerning the use of sticky, pheromone or scent traps are underway in several countries.

Coming on to the Polish market in recent times are offers from firms carrying out the “gel treatment” of trunks and claiming to an offer a two-year guarantee of effectiveness (Oszako 2004). These measures have been espoused and expounded vigorously by both the Polish media and the Nature Protection League (*Liga Ochrony Przyrody*). The period April-May 2003 saw chemical measures applied nationwide to combat the leaf-miner and leaf-blotch fungus simultaneously. These entailed the said gel treatment of some 60,000 trees in urban areas and parks. The process entails the infusion (i.e. the insertion into drilled holes) of a preparation known as ”gel for the simultaneous combating of the horse-chestnut leaf-miner and the fungus *Guignardia aesculi*”. Those who implement this method (also known as ”endotherapy”) suggest that it does not give rise to any pollution of the urban environment by plant protection agents, since the chemicals pass straight into trees. The other side of the coin is of course that the measure is highly invasive (damaging to trees), something that itself arouses considerable controversy. In consequence, in 2004, the Warsaw City Board commissioned research from Poland’s Forest Research Institute into the influence and effectiveness of the methods of combating the leaf-miner applied up to that time. In the 3 years of the grant period at least, the results obtained were indicative of a markedly negative influence of the infusion treatment on treated trees.

A REVIEW OF THE PROTECTION METHODS ON OFFER

The pest is very hard to combat, thanks to the dynamics of the leaf-miner population, the overall state of health of horse chestnuts and the binding formal/legal restrictions on the use of insecticides in urban areas. The currently pandemic nature of the horse-chestnut leaf-miner’s presence poses a fundamental problem when it comes to combating the species, as does the dispersed nature of the horse chestnut population. The result is the continued renewed infestation of trees. Measures to counteract the pest could involve the pupal, larval or adult stages.

1. The mechanical methods

Raking-up of leaf litter

The raking of litter results in the destruction of pupae (Głowacka 2005). The first (spring) generation of moths does not cause trees to shed their leaves, with the imago flying off from leaves remaining *in situ*. The pupal stage is shortlived, with the imago capable of flying off after just a few days, depending on the weather. However, some of the most weakened leaves do fall at this stage. It is important that falling leaves with second- (summer-) generation pupae be raked up quickly, since some of these will be within cocoons capable of overwintering.

The raking-up of litter containing third- (autumn-) generation pupae is of fundamental importance. The process must be systematic and scrupulous. Pupae fall of the brittle and decomposing leaves quite rapidly, then remaining beneath the tree until the time of the spring emergence.

While the raking of litter is both difficult and time-consuming (with leaves blowing around and shrubs or perennials present to hinder the process), it also serves the additional aim of limiting amounts of spores of the leaf-blotch fungus (Oszako 2004). Garden “vacuum cleaners” are employed effectively in these situations (Kosibowicz 2005). Equally, the leaves raked or sucked up in these ways will without question need burning once collected, since their composting would seem to be problematical.

Unfortunately, the removal of litter represents the transfer of valuable organic matter out of the system, though this can be seen as a calculated “necessary evil” where the growing of trees in urban areas or gardens is concerned. Perhaps more importantly, litter-removal also entails the departure of such allies in the counteraction of pests and diseases as natural predators and parasites.

Sticky traps

Sticky traps are placed on tree trunks (Plate 2). Their effectiveness is determined by the durability of the adhesive and the possibility for enhancement involving pheromones. Traps should be changed when become dry or full of insects, spiders and organic pollutions. Being not selective there are the subject of criticism by environmentalists.

2. Biological methods

Work on increasing the level of environmental resistance is underway and several antagonistic organisms are now known. In Poland their number is growing each year reaching now a level of 16 species of parazotoids. This figure is far however from Macedonia where the number of parazotoids is at least double. Anyway, it seems that their feasibility to reduce *Cameraria* population is around 1%. It means that biological methods so far remain a theoretical possibility only.

Plate 2. The sticky traps should cover as much of the trunk surface as possible

3. Chemical counteraction

This may only be applied in specific cases, e.g. where avenues or individual trees of heritage significance are concerned. Agents are used to combat the feeding larvae.

Larvicides hindering chitin production and hence confining larval development may be used. The agents in question often have partial ovicidal activity as well. Spraying may take place in the swarming/mating period, and hence in late April or early May, depending on the weather. The agent is stomach-acting and therefore needs to be used before larvae penetrate leaves. The method has been tried out in urban conditions using several different agents harmless to endotherms. The drawback is a failure to prevent protected leaves from being colonised by any third generation that may appear.

Counteraction using systemic agents injected into the soil and thus by definition of minimal soil mobility. Such measures need to be taken before leaves develop, but their effectiveness extends through the entire growing season. Their influence on biological life in soil is in question and therefore is the serious constrain for their application on a large scale.

Injections of "gel" formulations into the trunk, which should unfortunately be regarded as pointless. Countermeasures involving systemic agents assume that advantage will be taken of the active process by which water flows in several timber rings, the depth to which holes are drilled. However, this water current is only of limited effectiveness in a tree that is weakened, whether this be by leaf-miners, leaf-blotch fungus or above all the dry urban conditions and unfavourable weather. And the process is further impaired by the wounding that the drilling process induces. Furthermore, no account is taken of embolizm procesus (air stopper formation) i.e. the cutting off of capillary action once air enters vessels. And even if this is not the case, the required number of drilling episodes gives rise to non-permissible overall wounding of the tree.

Equally, the insecticides tried and tested in many countries may not be permitted for use in towns and cities, and so cannot be applied in pest countermeasures. Furthermore, even if a positive effect is achieved in a given year, the fear remains that leaves will simply be colonised once again in the following season. Thus the means of simultaneously counteracting the horse-chestnut leaf-miner and the fungal blight that are on offer in Poland will certainly require careful further observation.

THE NEGATIVE INFLUENCE OF THE SO-CALLED INJECTION (INFUSION) ON HORSE CHESTNUTS

This results from the way the measure is implemented, which is to say by the introduction of the "gel" into drill-holes of 8 mm in diameter to a depth of around 8 cm and at 15 cm intervals around the trunk (Siewniak *et al.* 2006). The treatment is applied in spring, prior to foliage development. The drill passes through the bark, bast wood, cambium and active wood. This leads to the appearance of numerous wounds that give rise to very unfavourable physiological processes and generally negative consequences (with up to 25 wounds on broader trees).

Methodology for studies on the reactions of horse chestnuts to infusions

Anatomical studies were carried out during the years 2004 and 2005 on horse chestnuts in urban or park conditions made subject to the gel treatment in springs. The trees in question were large and in the main with trunks showing signs of an advanced process of inner decay. The foliage of all the trees was dominated to varying degrees by larvae of the horse-chestnut leaf-miner. Samples of wood were taken using an increment borer, or else were prepared from trees already cut down and removed.

RESULTS

External and internal symptoms

All drilled holes had given rise to discharge, this mostly ceasing by the end of summer, though in some cases continuing into autumn (Plate 3). The discharge represents the draining off of the so-called "wet wood". It is watery, but contains compounds of a biostatic nature arising through a defensive mechanism on the part of the tree. Wound tissue was not noted around most of the holes drilled, though some holes did visibly possess a certain minimal amount of callusing. Only in the case of a single hole drilled in a young tree was a callus found to be entirely overgrowing a hole.

The wood around the drilled holes is wet and discoloured as a consequences of trees' defence reactions to wounding. Severe wetting of the wood ("wet wood") results from bacterial infection and the tree's attempt to safeguard itself against fungal infection. All living parenchyma cells die off throughout the area of wet wood. This process results in the generation of gases that encourage exudation from wounds and long-lasting external discharge. In spite of this, any protection offered is

Plate 3. Discharges on the bark can be toxic and contain exuded plant protection agents

shortlived. Once it has dried out (even in the same season), the wood in question is once again entirely vulnerable to further infection and is subject to bruising. The wood also turns brown. The first period after treatment sees yeasts and moulds encouraged, these paving the way for the fungi that give rise to timber decay (Oven and Levanic 2003). The size and shape of the area of timber discolouration and generation of the so-called protective wood is in line with the principle of the four walls and CODIT (the Compartmentalization of Decay In Trees), as proposed by Shigo in 1986. Wall ”1” provides quite effective limitation of the axial spread of rot upwards and downwards. Wall ”2” offers the least effective restriction through deprivation of energetic materials – a wound is hypothetically created on the core-wood side. Wall ”3” is a quite effective restricter of the spread of rot to adjacent areas, while wall “4” of the so-called barrier zone provides a very effective confinement of decay through the *de novo* creation of wood between the wound and the bast layer. A decaying trunk only has the remaining healthy wall of the trunk, which has to serve all physiological and static functions. All that is formed in unwounded areas in wall ”2”, which limits the centrifugal infection of the remaining healthy wall by fungi from a rotting interior.

The parts of the wood experiencing pathological changes as a result of drilling assume the shape of elongate, boat-shaped columns (with the drill-hole towards the interior) known as compartments. Within such compartments isolated by walls, the tree resigns from all physiological functioning. The extent of the visible changes can reach the following dimensions: lengthwise to up to 40 cm above and below the hole, laterally left and right up to 5 cm from the entrance and inwards several centime- tres from the base of the drill-hole.

If the drill passes through the wall of the healthy wood and breaks through wall ”4”, it gives rise to a typical compartment with all the walls present (Plate 4). In such a case the entire flow of discharge (probably together with the ”gel”) is in the direction of the decaying trunk interior.

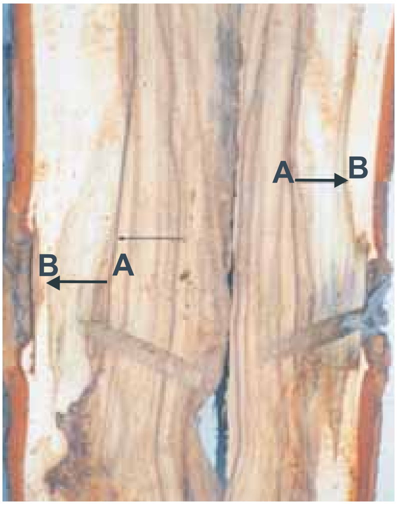


Plate. 4. Internal changes in the wood of gel-treated horse chestnuts: A wall ”4”, B wall ”4 bis”

Observed models for the reaction of trees to infusions

Model 1 – wall “4” is penetrated by the drill and discharge occurs into the decaying interior of the tree. There is then a clear development of a column of discoloured wood. This model arises in trees with extensive trunk rotting but adequate vitality.

Model 2 – wall ”4” is not cut through, so discharge to the outside takes place and there is clear development of a column of discoloured wood. This arises in young trees, or where the width of the remaining healthy wall exceeds the drilling depth. The tree has to show vitality if it is to react.

Model 3 – without the “wet wood” phase. This model reflects the lack of an appropriate reaction to wounding it what must in that case be a very much debilitated or weakened tree.

CONCLUSIONS

The discolouration of timber is an irreversible process, representing the first phase in its depreciation.

The horse chestnut has a very limited capacity to compartmentalise. Infusions are generally given to old, sick and weakened trees, most often growing in unfavourable environmental conditions. While the period before leaves develop is a choice justified by the requirements of the gel treatment, it is unsuitable from the point of view of tree physiology. Since trees are capable of generating reserve materials via the assimilation process, the best conditions under which they may bring protective and regenerative processes into effect following wounding are those present once the growing season has begun.

As all a tree’s protective and regenerative mechanisms require large supplies of energy, the effectiveness of these mechanisms declines in the event of a tree being debilitated. Thus a weakened horse chestnut is not able to implement effective compartmentalisation of the area of changed timber reflecting the wounding induced by drilling. Taken together, the drilling of some 20 holes in a single trunk – and the changes in wood this induces – constitute a major internal wounding event. On average, this may involve between 30 and 50% of the circumference of the white wood in the first two years. As individual internal wounding tends to spread out, there is reason to fear that wounds will link together to produce uniform internal wounding. Where the trunk is in decay, the narrow bands of wood that remain healthy can be entirely surrounded by a ring of timber undergoing depreciation processes. The rate at which this depreciation proceeds will depend on the condition of the tree, but the wood will become subject to rapid fungal rotting. At this point it will be necessary to anticipate, not only a restriction on all physiological functions in the trunk, but also timber decay and a compromising of the trunks mechanical durability.

Several issues remain to be accounted for. For example, how should gel-treated trees be proceeded with once the alleged two-year period of effectiveness of the measure has elapsed? At what rate does the timber depreciation process progress? To what extent do the plant protection agents reach the vessels and become subject to transport to the leaves? What is the influence of these chemicals on the wound, the timber depreciation process and the protective mechanisms? What happens with the insecticide-fungicide mixture? To what extent is it re-exuded from the drilled holes? And what is the influence of the chemical compounds being employed on any so-called ”environmental resistance” to the pest that may be developing?

The Polish literature still lacks basic information on the effectiveness of and responses to the application of the gel treatment on horse chestnuts. In other countries, the use of the infusion method in relation to old, sick and weakened horse chestnut trees was abandoned at the experimental stage. Certain positive effects noted for endotherapy are based on a process of so-called micro-injection taking an entirely different course, insofar as it is applied to young and relatively healthy horse chestnuts during their nursery production. Likewise, microinjection is applied prophylactically to healthy trees in the case of other diseases too.

Horse chestnuts made subject to the gel-treatment measure certainly require careful monitoring. This should relate in particular to trees' statics, and the surroundings potentially threatened by them.

References

Głowacka B. 2005: Usuwanie liści zasiedlonych przez szrotówka kasztanowcowiaczka *Cameraria ohridella* Deschka & Dimic jako metoda ochrony kasztanowca zwyczajnego *Aesculus hippocastanum* L. Leś. Pr. Bad., 2: 139-141.

Kosibowicz M. 2005: *Cameraria ohridella* Deschka & Dimic (Lepidoptera, Gracillaridae), nowy inwazyjny szkodnik kasztanowca białego *Aesculus hippocastanum* L. w Polsce – biologia i metody zwalczania. Leś. Pr. Bad., 2: 121-132.

Moreth L., Schoenitzer K., Diller E., Baur H. 2001: Die Überwiterungsraten der Rosskastanienminiermotte, *Cameraria ohridella* Deschka & Dimic, und ihrer Antagonisten. Jahrbuch der Baumpflege, 252-253.

Oszako T. 2004: Podstawy metodyczne do walki ze szrotówkiem kasztanowcowiaczkiem. Leś. Pr. Bad., 2: 130-132.

Oven P., Levanic T.: Jahrringanalytische und holzanatomische Untersuchungen unterschiedlich befallener Rosskastanien (*Aesculus hippocastanum)* in der Stadt Lublijana (Slowenien). Ibidem, 254-259.

Shigo A. L. 1986: A new Tree Biology. Facts, Phothos, and Philosophies on Trees and Their Problems and Proper Care. Shigo and Trees, Associates; Durham, New Hampshire.

Siewniak Marek., Oszako T., Siewniak Margarita 2006: Negatywne zmiany anatomiczne pnia powodowane przez próby zwalczania szrotówka kasztanowcowiaczka metodą "żelowania" . Biuletyn Polskiego Stowarzyszenia Pracowników Dezynfekcji, Dezynsekcji i Deratyzacji, 3/46: 26-31.

Tomicek Ch. 1998: Die Rosskastanien-*Miniermotte.* Ibidem, 136-143.

Tomicek Ch., Krehan H. 2001: Neue Erkenntnisse zur Rosskastanienminiermotte – Befallsdynamik und Bekämpfungsmöglichkeiten. Ibidem, 15-24.

EUCALYPTS AS SOURCE AND SINK FOR INVASIVE PESTS AND DISEASES

Simon A. Lawson

Department of Primary Industries & Fisheries Queensland, 80 Meiers Rd.,
Indooroopilly, Qld 4068, Australia.
e-mail: simon.lawson@dpi.qld.gov.au

INTRODUCTION

There are more than 700 species of eucalypts (Myrtaceae: Myrtales) in two genera, *Eucalyptus* and *Corymbia*. All of these are endemic to the Australian mainland, Tasmania and other coastal islands, with the exceptions of *Eucalyptus deglupta* and *E. urophylla*, which are native to Papua New Guinea and Timor, respectively. Eucalypts are by far the most dominant tree in the Australian landscape, and are adapted to a wide variety of climatic and site conditions, from deserts to rainforest fringes to above the snowline in the Australian Alps.

Australia's 164 million hectares of forests are dominated mainly by eucalypt forest, eucalypts excluded only from tropical and subtropical rainforest ecosystems. At present, eucalypt plantations in Australia make up less than 0.5% (715,000 ha) of the total forest area and the majority of these plantations are of a single species, *E. globulus*, mostly grown for pulp, although this is beginning to change as these plantations expand into subtropical and tropical Australia. Much of this plantation expansion is driven by the cessation of logging in native forests and the need to substitute this resource by wood products derived from plantations.

AN EXPORT SUCCESS STORY

Eucalypts were first encountered by Europeans in 1770 by the botanist Joseph Banks on Captain James Cook's voyage of discovery to the east coast of Australia, but generally they were considered a curiosity for many years by European settlers. Eventually characteristics such as fast growth and high quality, durable timber were recognised. One of the first champions/promoters of eucalypts was the botanist Baron Ferdinand von Mueller, who was responsible for sending seed to many parts of the world. Eucalypts have now been grown outside Australia for more than 200 years and are presently grown in over 80 countries worldwide on all continents excluding Antarctica.

WORLDWIDE GROWERS OF EUCALYPTS

There are currently more than 16 million hectares of eucalypts planted around the world for a large number of purposes including pulp for paper manufacture, solid wood and structural timbers, and as woodlots for fuel. The major growers of eucalypts by area planted are listed in Table 1. India has the largest area of eucalypt plantations, followed by Brazil and China. The recent expansion of plantations in Australia has seen the home of the eucalypt move up to fourth place in this list.

Table 1. Area of eucalypt plantations (thousend ha) by country for the top 13 countries worldwide

Country	Area (thousend ha)
India	8,000
Brazil	3,000
China	1,300
Australia*	720
South Africa	600
Portugal	590
Uruguay	500
Vietnam	450
Thailand	450
Chile	330
Argentina	300
Philippines	190
Indonesia	130
Total	16,560

Source: FAO Forest area statistics at year 2000 (http://www.fao.org/forestry/site/25630/en)
*Area at 2004 (National Forest Inventory 2005. National Plantation Inventory 2005 Update, Bureau of Rural Sciences, Canberra)

OTHER SUCCESSFUL AUSTRALIAN EXPORTS…

Along with the eucalypt trees, many associated insects have been exported widely around the world. Table 2 shows a sample of sixteen species which have become significant exotic pests of eucalypts overseas. The two cerambycid beetles *Phoracantha semipunctata* and *P. recurva* are the most widely distributed of these pests, now occurring on all continents except Antarctica. Their high level of invasiveness is probably due to their cryptic biology and the ability to survive in dried eucalypt timber as it is exported around the world. The defoliator and sap-sucker guilds are well represented in terms of numbers of species invading overseas, while gall insects have been prominent recent exports, with *Ophelimus* sp. and *Leptocybe invasa* particularly threatening to plantations in the Middle East, Europe, Africa and South America.

Table 2. Australian eucalypts as a source for invasive insect pests

INSECT PESTS	COUNTRIES ESTABLISHED
Stem borers	
Phoracantha semipunctata	South Africa, Zambia, USA, Argentina, Chile, Uruguay, Brazil, South Europe, Israel
Phoracantha recurva	South Africa, Zambia, USA, Argentina, Chile, Tunisia, Uruguay, Brazil?
Defoliators	
Gonipterus scutellatus	South Africa, USA, Chile, New Zealand
Paropsis charybdis	New Zealand
Trachymela tincticollis	South Africa
Phylacteophaga froggatti	New Zealand
Strepsicrates macropetana	New Zealand
Strepsicrates sp. nr. *semicanella*	China
Sap-suckers	
Glycaspsis brimblecombei	USA, Chile
Eucalyptolyma maideni	USA
Ctenarytaina eucalypti	New Zealand, Chile, Europe, Africa, India, Brazil
Eriococcus coriaceus	New Zealand
Thaumastocoris australicus	South Africa, Chile
Gallers	
Ophelimus eucalypti	New Zealand
Leptocybe invasa	Israel, North & East Africa, Thailand, China & Vietnam?
Ophelimus sp.	Israel, North Africa

TRENDS IN INTRODUCTIONS OF EUCALYPT INSECTS – TWO EXAMPLES

As is the case for many invasive forest species, the rate of spread of eucalypt insect pests around the world has increased markedly in recent years in association with the increase in volume of world trade. This has been particularly the case in New Zealand and California.

New Zealand

While New Zealand is not a major grower of eucalypts, eucalypts are a common urban amenity tree and plantations do form a small but important part of the timber industry. More than 26 species of specialist Australian eucalypt insects are now established in New Zealand and this number continues to grow (Withers 2001). From the early 1900's introductions of eucalypt associated insects from Australia averaged about one every seven years, but this accelerated to one every 17 months between 1980 and 2000.

USA (California)

For 150 years following their initial introduction to California, eucalypts were free of both insect pests and diseases, but in the last 20 years numerous herbivorous insect species have been introduced into the state and have caused significant damage (Paine and Millar 2002), including two stem borers and several psyllids.

EUCALYPTS AS A SINK: EXOTIC THREATS TO EUCALYPTS IN AUSTRALIA

There are at two particularly high-risk pathways for exotic eucalypt invasives to enter Australia: from the tropical north, and *via* the large-scale growers and exporters of eucalypt products. Another threat is posed by extreme generalist species such as Gypsy moth (*Lymantria dispar*) which has a well-documented potential pathway *via* imports from North-east Asia and the Russian Far East.

The threat from the north

The route with the highest risk is from South-east Asia & Papua New Guinea into tropical northern Australia, particularly via trade between the islands lying between these countries. An Australian Quarantine Inspection Service (AQIS) Pest Risk Analysis in 1998 prioritised 10 insects and 12 fungal pathogens that posed the greatest threat (see Table 3). Eight of these insects are endemics that have adapted to eucalypts in plantations outside Australia, with a further two being generalist termite species.

Table 3. Australian eucalypts as a sink for exotic insect pests

Pest	Country(ies) of origin	Damage
Amblypelta cocophaga (Hempitera: Coreidae)	Solomon Islands, PNG	Shoot dieback
Agrilus opulentus (Coleoptera: Buprestidae)	PNG	Tree girdling
Agrilus sexsignatus (Coleoptera: Buprestidae)	Phillipines	Tree girdling
Celosterna scabrator (Coleoptera: Cerambycidae)	India, Pakistan	Stem damage
Coptotermes curvignathus (Isoptera: Rhinotermitidae)	India, Myanmar, Malaysia, Singapore, Thailand, Vietnam, Cambodia, Philippines, Indonesia	Death of young trees
Coptotermes formosanus (Isoptera: Rhinotermitidae)	China, Taiwan, Japan, Hawaii, Micronesia, Sri Lanka, South Africa, USA, Pakistan, Brazil	Death of young trees
Helopeltis spp. (Hemiptera: Miridae)	Sri Lanka, India, Taiwan, Myanmar, Thailand, Vietnam, Malaysia, Sumatra, Java, Timor, Sulawesi, Kalimantan, Philippines, Saba, PNG, Australia, west Africa, Singapore, Bhutan	Wilt and tip dieback
Oxymagis horni (Coleoptera: Cerambycidae)	Solomon Islands, PNG	Branch & stem tunneling
Strepsicrates rothia (Lepidoptera: Tortricidae)	Sri Lanka, India, Mauritius, Nigeria, Pakistan, Malaysia	Defoliation, tip dieback
Zeuzera coffeae (Lepidoptera: Cossidae)	Sri Lanka, India, Malaysia, Thailand, Myanmar, Phillipines, PNG, Indonesia	Stem damage

THE BIG GROWERS/EXPORTERS – BRAZIL AS AN EXAMPLE

More than 231 species of insects in 62 families have been recorded on eucalypts in Brazil (Berti Filho 1981). Native vegetation in Brazil has many myrtaceous spp., increasing the risk of host switching to *Eucalyptus*. Legislation requirements that a certain proportion of native vegetation must be retained within eucalypt plantations, enhances the likelihood that endemic pests and diseases will adapt to the new host.

Case Studies of host switching/adaptation

(1) Eucalypt rust (Puccinia psidii)

Eucalypt rust is distributed in South and Central America where it is endemic on native Myrtaceae, particularly guava. It was first described from Brazil in 1884 and the first outbreak on eucalypts was recorded in 1973. *P. psidii* has been reported from 13 eucalypt hosts in South America. Until 2004 eucalypt timber could be imported from Brazil into Australia under permit. A shipment of Brazilian eucalypt timber into Australia in June 2004 was found to have *P. psidii* spores on plastic wrapping packaging and imports of eucalypt timber from guava rust reported countries have since been suspended.

(2) Cossid moths (Lepidoptera: Cossidae)

Quince borer (*Chilecomadia valdiviana*) is typically associated in Chile with *Salix chilensis* (Saliaceae) and commercial fruit, ornamental and some native forest species. In 1992 it was detected attacking *E. nitens* and is also occasionally found associated with *E. gunnii*, *E. camaldulensis* and *E. delegatensis* (Lanfranco and Dungey 2001). Another Quince borer *Coryphodema tristis*, which normally is recorded feeding on Vitaceae and other fruit trees such as quince in the Cape region in South Africa was found attacking *E. nitens* in 2004 (Gebeyehu *et al.* 2005). Both these species pose a potential risk to eucalypts in Australia and have been placed on the 'watch list' by the Office of the Chief Plant Protection Officer in Australia (see http://www.daff.gov.au/planthealth)

Generalists – Gypsy Moth

The Gypsy moth (*Lymantria dispar*) is able to complete development on 26 of 55 native Australasian tree species that it has been tested against. Eucalypts show considerable variation in host acceptability, but larval performance on five of the eucalypts tested was as good as the preferred native host species of *L. dispar* (*Quercus pubescens* and *Q. robur*) in Europe (Matsuki *et al.* 2001). Several interceptions of egg masses of *L. dispar* have been made at Australian ports over recent years and an early warning pheromone trapping program is carried out at a number of Australian ports to intercept this pest.

STRATEGIES FOR MINIMISING THE RISK

- Retain strong barrier quarantine & import regulation
- Promote capacity-building & collaboration on forest invasive species in the region and with major eucalypt growers such as South Africa and Brazil.

- Strengthen quarantine surveillance in known high-risk entry points (e.g through northern Australia)
- Increase capacity in early warning/rapid response capability.
- Maintain capability in forest health surveillance.
- Develop screening programs to identify resistant/tolerant germplasm for plantation species.

Acknowledgement. *Grateful thanks go to to Forestry Plantations Queensland who supported the author's attendance at the inaugural IUFRO working party S7.03.12 meeting "Alien invasive species and international trade", held in Radom, Poland 3 –7 July 2006.*

References

Berti Filho E. 1981. Coleoptera of forestry importance. 1. Scolytidae. IPEF. 1979, recd, 39-43.

Gebeyehu S., Hurley B. P., Wingfield M. J. 2005. A new lepidopteran insect pest discovered on commercially grown *Eucalyptus nitens* in South Africa. South African Journal of Science, 101: 26-28.

Lanfranco D., Dungey H. S. 2001. Insect damage in *Eucalyptus*: a review of plantations in Chile. Austral Ecology, 26/5: 477-481.

Matsuki M., Kay M., Serin J., Floyd R., Scott J. K. 2001. Potential risk of accidental introduction of Asian gypsy moth (*Lymantria dispar*) to Australasia: effects of climatic conditions and suitability of native plants. Agricultural and Forest Entomology, 3: 305-320.

Paine T. D., Millar J. G. 2002. Insect pests of eucalypts in California: implications of managing invasive species. Bulletin of Entomological Research, 92: 147-151.

Withers T. M. 2001. Colonization of eucalypts in New Zealand by Australian insects. Austral Ecology, 26/5: 467-476.

DETECTION OF WOOD-BORING SPECIES IN SEMIOCHEMICAL-BAITED TRAPS

Jonathan Sweeney[1], Peter de Groot[2], Leland Humble[3], Linda MacDonald[2],
Jessica Price[1], Tomasz Mokrzycki[4], Jerzy M. Gutowski[5]

[1]Canadian Forest Service, Natural Resouce Canada, Fredericton NB
[2]Canadian Forest Service, Natural Resouce Canada, Sault Ste. Marie ON
[3]Canadian Forest Service, Natural Resouce Canada, Victoria BC
[4]Department of Forest Protection and Ecology, Faculty of Forestry,
Warsaw Agricultural University, Poland
[5]Forest Research Institute, Department of Natural Forests, 17-230 Bialowieza, Poland

We need effective, practical survey and detection tools for early detection of invasive species, to assist in delimiting the area of recently established pest populations once they are detected, and for monitoring the progress of eradication and containment programs. Sex and aggregation pheromones are great for species-specific detection at low densities, and the obvious lure choice for monitoring an established invasive or determining pest-free status. However, insect communication and host-finding behaviours are poorly understood for many species of wood-boring beetles and there is a limit to the number of species-specific lures/traps that can be used in operational surveys.

Host volatiles may be less effective than pheromones at detecting low-density populations of a particular species, but can attract (detect) a large number of different species. For example, α-pinene is attractive to many species of coniferous bark- and wood-borers, including the cerambycids (*Asemum striatum* (L.)*, Arhopolus rusticus nubilus* (LeConte)*, Monochamus titillator* (F.)*,* and *Xylotrechus integer* (Hald.); the scolytines (*Hylastes ater* (Payk.)*, Ips grandicollis* (Eichhoff)*, Tomicus minor* (Hartig)*,* and *T. piniperda* (L.); the buprestid (*Buprestis lineata* F.), and the predators *Enoclerus nigripes rufiventris* (Spinola), *E. nigrifrons gerhardi* Wolcott*,* and *Thanasimus formicarius* (L.) (Cleridae) (Pertunnen 1957, Byers *et al.* 1985, Schroeder 1988; Chénier & Philogčne 1989; Miller 2006; Allison *et al.* 2004). Ethanol, often produced in stressed or diseased trees, is attractive to several scolytines, e.g., *Trypodendron lineatum* (Oliv.)*, T. domesticum* (L.)*, Gnathotrichus sulcatus* (LeConte), *Xyleborus saxeseni* (Ratz.), *X. affinis* Eichhoff*, Hylurgops palliatus* (Gyll.)*,* and curculionids, e.g., *Hylobius abietis* (L.) (Moeck 1970; Schroeder 1988; Salom & McLean 1990; Byers 1992; Lindelöw *et al.* 1993). Some species are more attracted to a blend of monoterpenes than to either α-pinene or ethanol alone, e.g., *Xylotrechus undulatus* (Say)*, Tetropium fuscum* (F.)*,* and *Monochamus urussovii* (Fischer) (Cerambycidae) (Sweeney *et al.* 2004, 2006) and *Hylastes ater, Ips grandicollis* (Eichhoff), and

Tomicus minor (Scolytinae) (Schroeder 1988; Chénier & Philogčne 1989). Furthermore, attraction of many species is synergized by a combination of ethanol plus α-pinene or ethanol plus a blend of monoterpenes, e.g., *Monochamus scutellatus scutellatus* (Say)*, M. notatus* (Drury)*, Xylotrechus longitarsis* Casey*, Tetropium fuscum, T. castaneum* (L.)*, T. cinnamopterum* Kirby*, Spondylis buprestoides* (Cerambycidae) (Allison *et al.* 2001; Morewood *et al.* 2002; Sweeney *et al.* 2004), *Hylobius pales* (Herbst)*, H. abietis, H. pinastri* (Gyll.) (Curculionidae), *Dryocoetes autographus* (Ratz.)*, T. piniperda* (but too much ethanol repels), *Hylastes cunicularius* Erichson*, H. opacus* Erichson*, H. brunneus* Erichson (Scolytidae), and the clerid, *Thanasimus dubius* (F.) (Tilles *et al.* 1986; Vité *et al.* 1986; Schroeder 1988; Chénier & Philogčne 1989; Lindelöw *et al.* 1993; Miller 2006). Finally, some cerambycids are attracted to bark beetle pheromones (e.g., ipsenol, ipsdienol, MCH, and frontalin) or pheromones plus monoterpenes plus ethanol, e.g., *Monochamus clamator* (LeConte)*, M. s. scutellatus, M. titillator* (F.)*, M. obtusus* Casey*,* and *M. notatus* (Drury) (Billings & Cameron 1984; Allison *et al.* 2001, 2003; de Groot & Nott 2004).

Lindgren funnel traps baited with either: 1) α-pinene + ethanol (for woodborers of conifers); 2) ethanol (for woodborers of deciduous hosts); or 3) the 3-component *Ips* lure consisting of *cis*-verbenol, 2-methyl-3 buten-2-ol, and racemic ipsdienol (targeting *Ips typographus* (L.)*, I. sexdentatus* (Börner), *Hylurgus ligniperda* (F.)*,* and *Orthotomicus erosus* (Woll.) have been used by the Canadian Food Inspection Agency (CFIA) and the USDA Forest Service and Animal and Plant Health Inspection Service (APHIS) for several years. These traps have provided the first records of established populations of several species introduced to North America, including *Hylurgops palliatus* (2001, α-pinene+ethanol), *Arhopalus pinetorum* (Woll.) (2001, α-pinene+ethanol), *Xyleborus similus* Ferrari (2002, ethanol), *X. glabratus* Eichhoff (2002, ethanol), *Scolytus schevyrewi* Semenov. (2003, α-pinene+ethanol), *Sirex noctilio* F. (2005, *Ips* lure), as part of the USDA FS and APHIS rapid detection pilot program or APHIS-Cooperative Agricultural Pest Surveys (CAPS) (Duerr 2005; Hoebeke & Acciavatti 2006; Rabaglia 2005). However, traps baited with these standard lures did not detect every forest insect species recently introduced to North America; notable exceptions include the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (ALB), brown spruce longhorn beetle, *T. fuscum,* and the emerald ash borer, *Agrilus planipennis* Fairmaire (EAB). Both the ALB and EAB were first detected in New York and Michigan, respectively, on or near infested host trees (Haack *et al.* 1996; McCullogh & Katovich 2004). The earliest recorded collection of *T. fuscum* in Canada actually was in volatile-baited traps (in 1990 in Point Pleasant Park, Halifax, Nova Scotia on stickem-coated stovepipe traps baited with turpentine (Robertson 1990) but the specimens were misidentified as the nearctic *T. cinnamopterum* and *T. fuscum* was not actually detected in Halifax until 1999, when it was reared from bolts cut from infested spruce trees (Smith & Hurley 2000).

The most effective lure to date for *T. fuscum* is a blend of monoterpenes (spruce blend) plus ethanol released at ultra high rates (UHR) (Sweeney *et al.* 2006) and it attracts mainly *Tetropium* spp., at least among the cerambycids species trapped in Halifax, Canada and Białowieża, Poland. Of 41 species of cerambycids trapped in

Halifax and Poland in 2002 and 2003, 74% to 89% of specimens were *Tetropium* spp. caught in spruce blend plus ethanol-baited traps; *Tetropium* accounted for only 0% to 11% of cerambycid specimens captured in unbaited traps (Sweeney *et al.* 2004, 2006).

To determine whether the combination of spruce blend plus ethanol and similar lures might be useful for detecting scolytines, we recorded the scolytine species captured in trapping experiments (for *T. fuscum*) conducted in Halifax in 2003 and Poland in 2005. The forest in Halifax was dominated by white spruce, *Picea glauca* (Moench) Voss., red spruce, *P. rubens* Sarg., and balsam fir, *Abies balsamea* L.. In Białowieża, Poland, the experiment was conducted in an old growth mixed deciduous forest with the most common species being Norway spruce, *Picea abies* L. Karst., English oak, *Quercus robur* L., European hornbeam, *Carpinus betulus* L., Scots Pine, *Pinus sylvestris* L., aspen, *Populus tremula* L., and European silver birch, *Betula pendula* Roth. In Halifax, cross-vane pan traps baited with five different combinations of spruce blend and ethanol at high or low release rates plus an unbaited control captured 19 different scolytine species (Table 1). The combinations of spruce blend (UHR or low release rate) plus ethanol (UHR) were significantly attractive to four species including *Trypodendron lineatum, Dendroctonus rufipennis* (Kirby), and

Table 1. Total numbers of Scolytine species captured in cross vane pan traps baited with five different combinations of spruce blend plus ethanol, at low or ultra high release rates, or an unbaited control trap, in a spruce dominated forest on McNabs Island, Halifax, Nova Scotia, Canada, in 2003

Species	Numbers captured
Cryphalus ruficollis Hopkins	15
Crypturgus pusillus (Gyllenhal)	5
Dendroctonus rufipennis (Kirby)	213
Dryocoetes affaber (Mannerheim)	49
Dryocoetes autographus (Ratz.)	48
Gnathotrichus materiarius (Fitch)	101
Hylastes porculus Erichson	9
Hylurgops pinifex (Fitch)	3
Ips borealis Swaine	5
Ips pini (Say)	4
Orthotomicus caelatus (Eichhoff)	10
Pityophthorus ramiperda Swaine	81
Pityophthorus sp. Eichhoff	1
Polygraphus rufipennis (Kirby)	23
Trypodendron lineatum (Oliv.)	381
Trypodendron retusum (LeConte)	1
Xyleborus dispar (F.)	69
Xyleborus sayi (Hopkins)	24
Xyloterinus politus Say	12

Table 2. Total numbers of Scolytine species captured in intercept traps baited with seven different host volatile lures at ultra high release rates or an unbaited control, in an old growth mixed deciduous forest with Norway spruce, English oak, Scots pine, European hornbeam, aspen and European silver birch near Białowieża, Poland, 2005. The lures were composed of blends of monoterpenes simulating those emitted from red spruce, alone or combined with ethanol

Species	Numbers captured
Crypturgus cinereus (Herbst)	84
Crypturgus hispidulus Thoms.	1
Dryocoetes autographus (Ratz.)	537
Hylastes angustatus (Herbst)	3
Hylastes ater (Payk.)	222
*Hylaste*s *cunicularius* Erichson	2317
Hylastes opacus Erichson	143
Hylurgus ligniperda (F.)	3
Ips typographus (L.)	192
Ips sexdentatus (Börner)	1
Orthotomicus laricis (F.)	2
Pityogenes chalcographus (L.)	30
Tomicus piniperda (L.)	16
Trypodendron lineatum (Oliv.)	1
Xyleborinus saxeseni (Ratz.)	11
Xyleborus dispar (F.)	51
Xyleborus monographus (F.)	3

Gnathotrichus materiarius (Fitch). In 2005 we varied the ratios of monoterpenes in the spruce blend lures to simulate those emitted from stressed (girdled) vs. unstressed red spruce trees and tested 6 different lures or lure combinations plus an unbaited control in, black IPM intercept traps (Advanced Pheromone Technologies, Marylhurst, OR) in Białowieża. A total of 17 different scolytine species were captured (Table 2), with five species (*Hylastes cunicularius, H. opacus, H. ater, Dryocoetes autographus, Crypturgus cinereus* (Herbst)) significantly attracted to unstressed spruce blend plus ethanol (both UHR lures).

In 2006, we initiated some field trapping trials in Canada (Vancouver, Toronto, and Halifax) to test whether we could improve the suite of lures for the detection of potentially invasive wood/bark-boring beetles. Specifically, our objectives were to test a series of 7 lures (including the three standards) plus an unbaited control to determine which lures were significantly attractive to wood-boring species, to compare lures for total number of species detected, and to determine the minimum number of traps required to detect most of the (significantly attracted) species in an area. Processing of catches has begun and one of the first points to emerge is that certain species, e.g., *Trypodendron lineatum,* are flooding the traps and slowing down the work of species sorting and identification. A repellent that deterred the catch of very common native species without disrupting the attraction of target species would be very useful, if such exists. It would be useful to repeat these experiments in coniferous forests in Europe and China.

In summary, we need a practical and effective suite of lures/traps to assist in early detection of invasive species. Host volatiles are significantly attractive to a range of wood-boring beetles, and can be surprisingly selective, but will never be as sensitive as a pheromone-based lure. Slight changes in terpene ratios can significantly affect attraction of some species. Our experiments will hopefully lead to improvements in invasive wood-borer surveillance and detection.

***Acknowledgments**. We thank the Canadian Forest Service, Canadian Food Inspection Agency, Forest Protection Limited, New Brunswick Dept. Natural Resources & Energy, Ontario Ministry of Natural Resources, Nova Scotia Dept. of Natural Resources and Halifax Regional Municipality for supporting this research, and Nichole Braun, Katie Burgess, Gregg Cunningham, Art Doane, Nelson Carter, Bob Guscott, Glenn Harrison, Ed Kettela, Troy Kimoto, Karen O'Leary, Wayne MacKay, Lee Mailman, Chris McQuarrie, Taylor Scarr, Dave Seaboyer, Georgette Smith, Krzysztof Sućko and Tom Walsh for logistical or technical assistance.*

References

Allison J. D., Borden J. H. , McIntosh R. L., de Groot P., Gries. R. 2001. Kairomonal response by four *Monochamus* species (Coleoptera: Cerambycidae) to bark beetle pheromones. J. Chem. Ecol., 27: 633-646.

Allison J. D., Morewood W. D. , Borden J. H. , Hein K. E., Wilson I. M. 2003. Differential bio-activity of *Ips* and *Dendroctonus* (Coleoptera: Scolytidae) pheromone components for *Monochamus clamator* and *M. scutellatus* (Coleoptera: Cerambycidae). Environ. Entomol., 32: 23-30.

Allison J. D., Borden J. H., Seybold S. J. 2004. A review of the chemical ecology of the Cerambycidae (Coleoptera). Chemoecology, 14: 123–150.

Billings R. F., Cameron R. S. 1984. Kairomonal responses of Coleoptera, *Monochamus titillator* (Cerambycidae), *Thanasimus dubius* (Cleridae), and *Temnochila virescens* (Trogossitidae), to behavioural chemicals of southern pine beetles (Coleoptera: Scolytidae). Environ. Entomol., 13: 1542-1548.

Byers J. A., Lanne B. S., Schlyter F., Löfqvist J., Bergström G. 1985. Olfactory recognition of host-tree susceptibility by pine shoot beetles. Naturwissenschaften, 72: 324-326.

Byers J. A. 1992. Attraction of bark beetles, *Tomicus piniperda, Hylurgops palliatus,* and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes. J. Chem. Ecol., 18: 2385-2402.

Chénier J. V. R., Philogčne B. J. R. 1989. Field responses of certain forest Coleoptera to conifer monoterpenes and ethanol. J. Chem. Ecol., 15: 1729–1745.

de Groot, P., and R. Nott. 2004. Response of the whitespotted sawyer beetle, *Monochamus s. scutellatus* and associated woodborers to pheromones of some *Ips* and *Dendroctonus* bark beetles. J. Appl. Entomol. 128: 483-487.

Duerr D. A. 2005. Early detection and rapid response pilot project. pp. 16-17 *in*: Gottshalk, K.W., ed. Proceedings, 16th U.S. Department of Agriculture Interagency research forum on gypsy moth and other invasive species 2005; 2005 January 18-21; Annapolis, MD. Gen. Tech. Rep. NE-337. Newtown Square, PA. USDA Forest Service, Northeastern Research Station. 107 pp.

Haack R. A., Cavey J. F., Hoebeke E. R., Law K. R. 1996. *Anoplophora glabripennis*: a new tree-infesting exotic cerambycid invades New York. Newsletter of the Michigan Entomological Society, 41(2-3): 1-3.

Hoebeke E. R., Acciavatti R. E. 2006. *Hylurgops palliatus* (Gyllenhal) (Coleoptera: Curculionidae: Scolytinae), an Eurasian bark beetle new to North America. Proc. Entomol. Soc. Wash., 108: 267-273.

Lindelöw A., Eidmann H. H., Nordenhem H. 1993. Response on the ground of bark beetle and weevil species colonizing conifer stumps and roots to terpenes and ethanol. J. Chem. Ecol., 19: 1393-1403.

McCullough D.G., Katovich S. A. 2004. Pest Alert: Emerald Ash Borer. USDA Forest Service State and Private Forestry Northeastern Area, Newtown Square, PA. NA-PR-02-04.

Moeck H. A. 1970. Ethanol as the primary attractant for the ambrosia beetle *Trypodendron lineatum* (Coleoptera: Scolytidae). Can. Entomol., 102: 985-995.

Miller D. R. 2006. Ethanol and (–)-α-Pinene: Attractant kairomones for some large wood-boring beetles in southeastern USA. J. Chem. Ecol., 32: 779-794.

Morewood W. D., Simmonds K. E., Wilson I. M., Borden J. H., McIntosh R.L. 2002. α-Pinene and ethanol: key host volatiles for *Xylotrechus longitarsis* (Coleoptera: Cerambycidae). J. Entomol. Soc. Brit. Columbia, 99: 117-122.

Perttunen V. 1957. Reactions of two bark beetle species, *Hylurgops palliatus* Gyll. And *Hylastes ater* Payk. (Col. Scolytidae) to the terpene α-pinene. Ann. Entomol. Fenn., 23: 101-110.

Rabaglia R. J. 2005. Exotic scolytids in North America: Who's here and what are the impacts. p. 67 *in:* Gottshalk, K.W., ed. Proceedings, XV U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species 2004; 2004 January 13-16; Annapolis, MD. Gen. Tech. Rep. NE-332. Newtown Square, PA. USDA Forest Service, Northeastern Research Station. 98 pp.

Roberston S. 1990. Point Pleasant Park Halifax, Nova Scotia bark beetle survey. Halifax Field Naturalists Report to the Technical Advisory Committee of the Point Pleasant Park Commission. 18 pp.

Salom S. M., McLean J. A. 1990. Flight and landing behavior of *Trypodendron lineatum* (Coleoptera: Scolytidae) in response to different semiochemicals. J. Chem. Ecol., 16: 2589-2604.

Schroeder L. M. 1988. Attraction of the bark beetle *Tomicus piniperda* and some other bark- and wood-living beetles to the host volatiles α-pinene and ethanol. Entomol. Exp. Appl., 46: 203-210.

Smith G., Hurley J. E. 2000. First North American record of the Palearctic species *Tetropium fuscum* (Fabricius) (Coleoptera: Cerambycidae). Coleopt. Bull., 54: 540.

Sweeney J., de Groot P., MacDonald L., Smith S., Cocquempot C., Kenis M., Gutowski J.M. 2004. Host volatile attractants for detection of *Tetropium fuscum* (F.), *Tetropium castaneum* (L.), and other longhorned beetles (Coleoptera: Cerambycidae). Environ. Entomol., 33: 844–854.

Sweeney J., Gutowski J. M., Price J., de Groot P. 2006. Effect of semiochemical release rate, killing agent, and trap design on capture of *Tetropium fuscum* (F.), and other longhorn beetles (Coleoptera: Cerambycidae). Environ. Entomol., 35: 645-654.

Tilles D. A., Sjodin K., Nordlander G., Eidmann H.H. 1986. Synergism between ethanol and conifer host volatiles as attractants for the pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae). J. Econ. Entomol., 79: 970-973.

Vité J. P., Volz H. A., Paiva M. R. 1986. Semiochemicals in host selection and colonization of pine trees by the pine shoot beetle *Tomicus piniperda.* Naturwissenschaften, 73: 39-40.

DO INSECTS INFEST WOOD PACKING MATERIAL WITH BARK FOLLOWING HEAT-TREATMENT?

Robert A. Haack[1], Toby R. Petrice[1], Pascal Nzokou[2], D. Pascal Kamdem[2]

[1]USDA Forest Service, Northern Research Station, 1407 S. Harrison Road, East Lansing, Michigan 48823, USA
[2] Michigan State University, Department of Forestry, East Lansing, Michigan 48824, USA

Exotic forest pests threaten forest resources in all countries. As a result of international trade, many bark- and wood-infesting insects are inadvertently transported in wood packing materials (WPM) such as crating, dunnage, and pallets. In the continental United States (USA), 25 new species of bark- and wood-boring beetles were discovered during 1985–2005 (Haack 2001, 2006). WPM is the suspected pathway for most of these beetles, although the exact mode of entry into the USA is unknown. As an example of the worldwide movement of insects in WPM, Figure 1 shows the countries of origin for bark- and wood-infesting beetles that were intercepted at ports of entry in the USA during the years 1985–2000. Overall, 8341 interceptions were reported during that 16-year period, representing 113 different countries of origin (Haack 2006). The top 10 countries of origin were, in decreasing order, Italy, Germany, China, Spain, Belgium, Russia, India, Mexico, France, and United Kingdom (Haack 2006). Similarly, in the case of Chile during the period 1995–1999, the top 10 countries of origin for intercepted wood-associated insects were USA, Brazil, Germany, Argentina, Italy, India, Peru, Colombia, Ecuador, and Spain. (Beeche-Cisternas 2000, Haack 2006).

Figure 1. World map depicting 113 countries (with stars) from which bark- and wood-infesting beetles were intercepted at ports of entry in the USA during the years 1985–2000 (Source: USDA APHIS, Port Information Network database; see details in Haack 2006)

In recognition that WPM is an important pathway for the movement of pests, the world community approved new international standards for WPM in 2002 (FAO 2002). These standards are often referred to as ISPM-15 (ISPM = International Standards for Phytosanitary Measures). The principal objective of ISPM-15 is to reduce the risk of introducing pests associated with WPM. Current ISPM-15 standards for WPM allow for the presence of bark when the wood is either heat-treated or fumigated with methyl bromide according to approved protocols. ISPM-15 also requires that all WPM used in international trade be marked to indicate the country of origin, the type of treatment used, and the company that conducted the treatment (FAO 2002). ISPM-15 has been implemented by many countries worldwide. The USA began enforcement of ISPM-15 in 2006.

The "International Forest Quarantine Research Group" or IFQRG (http://www.forestry-quarantine.org) was initiated in 2003. One of the principal objectives of IFQRG is to conduct research that addresses important questions related to ISPM-15. The first research question that IFQRG considered was: Can insects and disease organisms infest wood after treatment, especially when bark is present?

We initiated studies in 2004 and 2005 to evaluate post-treatment insect colonization of logs (2004) and lumber (2005) with varying amounts of bark. In June 2004, we used freshly cut 1-m-long logs from four species of trees, including *Acer rubrum* (red maple), *Carya glabra* (pignut hickory), *Pinus resinosa* (red pine), and *Quercus rubra* (northern red oak). All bark was retained on the logs. The logs were either heat treated according to ISPM-15 standards (56°C core temperature for 30 minutes) or left as untreated control logs. The logs were treated and placed in the field within 2 weeks of cutting. The logs were elevated a few centimetres above the ground and placed along a forest edge at the W.K. Kellogg Experimental Forest of Michigan State University near Augusta, Michigan, USA. Half of the logs were recovered about a month later (July 2004) and the remaining logs were recovered the following spring (March 2005). Each log was then cut in half: one half was dissected immediately and the other half was placed in a rearing tube to allow insects to emerge. All insects were collected, identified, and counted. The bark surface area of each log was calculated from its length and diameter measurements.

Overall, bark- and wood-boring insects (primarily Cerambycidae and Scolytidae) colonized and successfully reproduced in all treated and untreated logs of all four tree species tested. In fact, mean emergence densities were usually significantly higher on the heat-treated logs compared with the untreated control logs. For example, considering the pine logs, mean emergence density of scolytid bark beetles (*Ips* and *Orthotomicus* spp.) was 1352 adults/m^2 on heat-treated logs compared with 430 adults/m^2 on control logs. One interesting observation was that *Orthotomicus* adults were the dominant bark beetles to emerge from heat-treated pine logs while *Ips* adults dominated on the control pine logs. The genera of scolytids that were reared from the different tree species included *Gnathotrichus, Ips, Monarthrum, Orthotomicus, Xylosandrus,* and *Xyloterinus.* Similarly, the genera of reared cerambycids included *Acanthocinus, Monochamus, Saperda, Urographus,* and *Xylotrechus.*

In July 2005, using freshly cut red pine trees, we prepared about 200 1-m-long boards with varying amounts of bark along one edge. There were two board thickness categories: 1 inch (ca. 2.5 cm) and 4 inches (ca. 10 cm); and four bark categories: (1) no bark retained, (2) eight small (ca. 25 cm^2) bark patches, (3) two large (ca. 100 cm^2) bark patches, and (4) all bark retained. Half of the boards were heat treated (56°C core temperature for 30 minutes) and the remainder served as untreated controls. The boards were exposed to natural insect attack in a red pine stand at the Kellogg Experimental Forest by stacking them in piles in which all treatments were represented in each pile. The boards were retrieved from the field after 23 days and taken to the laboratory. Half the boards were dissected and half were placed in rearing tubes.

Overall, bark beetles in the family Scolytidae and borers in the family Cerambycidae colonized and oviposited in all treatments except those where all bark was removed. The bark beetles included species of *Ips* and *Orthotomicus* and the cerambycids included species of *Monochamus*. The bark beetles and cerambycids did not attack the bark-free boards or the bark-free areas of those boards with bark patches. Although bark beetle and cerambycid eggs were laid in the smallest (25 cm^2) bark patches, complete larval development did not occur. Complete bark beetle and cerambycid development, however, did occur on the larger (100 cm^2) bark patches. Again, average attack densities tended to be higher on the heat-treated boards compared with the control boards.

Although our studies indicated that borers will infest barked surfaces of WPM after heat treatment under ideal field conditions, it was not known to what degree treated WPM currently used in international trade contains bark, and how often live borers are associated with the bark.

To address this question, the first two authors (RAH and TRP) coordinated surveys of WPM at six US ports in summer 2006. The surveys were conducted in cooperation with the US Department of Homeland Security, Customs and Border Protection (CBP). Surveys were conducted at the ports of Detroit, Michigan; Elizabeth, New Jersey; Laredo, Texas; Long Beach, California; Savannah, Georgia; and Seattle, Washington. Typically, one CBP Agricultural Officer was assigned to conduct the survey at each port for a period of two weeks. R.A. Haack and T.R. Petrice provided training in the survey methods to the CBP inspectors and analyzed the survey results. The surveys were conducted as part of normal port operations and examined only WPM (crating, dunnage, and pallets) that had the ISPM-15 mark. The surveys consisted of inspecting the WPM for bark, measuring the size of the bark patches, and determining if live insects were present under the bark.

The first portion of the survey addressed the question: What is the incidence of bark on marked WPM and what sizes of bark occur? The largest patch of bark found on each WPM item (one entire pallet, or all the crating associated with a single product, or a single piece of dunnage) was assigned to one of four categories: (1) no bark found, (2) smaller than a credit card (ca. 45 cm^2), (3) larger than a credit card but smaller than a piece of paper (ca. 600 cm^2), and (4) larger than a piece of paper. Overall, of 5945 WPM items were inspected at the six ports, 5385 (90.6%) had no bark, 317 (5.3%) had bark that was smaller than a credit card, 232 (3.9%) had bark that was larger than a credit card but smaller than a piece of paper, and 11 (0.2%) had bark that was larger than a piece of paper. The shipments had originated from 50 different countries.

The second portion of the study addressed the question: What is the incidence of live insects (of quarantine significance) under bark on WPM that has the ISPM-15 mark? Overall, 563 WPM items with bark were inspected for live insects. These WPM items originated from 26 different countries. Live borers were found under bark on 7 of the 563 WPM items inspected (1.2%), including two WPM items with bostrichids, one with cerambycids, and four with scolytids. The seven WPM items with live borers were detected at two ports (Long Beach, CA and Savannah, GA) and all had originated from one single country. It was not possible to determine with certainty if the presence of live borers was the result of attack prior to ISPM-15 treatment (suggesting a failure of treatment) or after treatment (suggesting post-treatment colonization).

Based on the survey results, it is estimated that about 0.11% of all marked WPM entering the USA harbored live borers. This value was calculated by multiplying the percentage of WPM that had bark (9.4%; 560/5945) by the percentage of WPM with bark that harbored live borers (1.2%; 7/563).

The results of surveys and studies like those described above will allow regulators to judge the relative risk of bark associated with WPM and whether there is justification to require that WPM be manufactured from bark-free wood.

References

Beeche-Cisternas M. A. 2000. Riesgos cuarentenarios de insectos asociados a embalajes de madera y maderas de estiba de cargas de internacion en Chile. [In:] Proceedings: International Conference on Quarantine Pests for the Forestry Sector and their Effects on Foreign Trade, 27–28 June 2000, Concepcion, Chile. Concepcion, Chile: CORMA.

FAO (Food and Agriculture Organization), 2002. International standards for phytosanitary measures: guidelines for regulating wood packaging material in international trade. Rome, Italy: Food and Agriculture Organization of the United Nations, Pub. No. 15.

Haack R. A. 2001. Intercepted Scolytidae (Coleoptera) at U.S. ports of entry: 1985–2000. Integrated Pest Management Reviews, 6: 253-282.

Haack R. A. 2006. Exotic bark and wood-boring Coleoptera in the United States: recent establishments and interceptions. Canadian Journal of Forest Research, 36: 269-288.

ISPM15 TREATMENTS AND RESIDUAL BARK: HOW MUCH BARK MATTERS IN RELATION TO FOUNDER POPULATIONS OF BARK AND WOOD BORING BEETLES?

Hugh F. Evans

Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH
hugh.evans@forestry.gsi.gov.uk

INTRODUCTION

International movement of pests and pathogens is associated with trade in wood or wood products and there are many examples of the establishment of dangerous organisms and associated ecosystem damage in new countries (Liebhold *et al.* 1995). This risk is recognised globally and measures, at country and regional levels, are in place to try to reduce the likelihood of pests and pathogens successfully proceeding along pathways to establish in new locations. These measures are coordinated through the International Plant Protection Convention (IPPC) and promulgated through regional plant protection organisations. A number of pathways have been recognised as posing risks of pest movement and the two most dangerous are regarded as wood packaging and plants for planting.

WOOD PACKAGING AS A PATHWAY

The introduction of measures to treat packaging wood to kill pests and pathogens of quarantine concern is reducing the risks from this dangerous pathway. International Standards for Phytosanitary Measures (ISPM) Number 15, through the auspices of IPPC, is now in place for many countries globally and will, over time, be introduced for all wood packaging in international trade. Current measures to kill pests include heat treatment (HT) to reach a core temperature in the wood of 56°C for 30 minutes or fumigation with methyl bromide (MeBr) to a set schedule.

There is no doubt that these measures kill the great majority of pests that might be associated with the packaging wood. However, through the International Forestry Quarantine Research Group (IFQRG; www.forestry-quarantine.org), scientists in Germany, Canada, USA and UK have carried out research on the efficacy of the ISPM15 measures in relation to the risks arising from residual bark on the treated wood. Although the ISPM15 measures kill the pests already present, there is still a possibility that further infestation could take place between the time of treatment and final exportation from the country. All the research groups reported that residual bark remains suitable for infestation.

IMPLICATIONS FOR CURRENT ISPM15 STIPULATIONS

Currently, ISPM15 measures concentrate on active treatment to kill pests in packaging wood, but there is a debate, particularly within the European Union, on whether removal of bark to prevent post-treatment infestation should also be required. ISPM15 states that "Subject to technical justification, countries may require that imported wood packaging material subjected to an approved measure be made from debarked wood". The issue of technical justification is still being investigated and can be split into two main questions:

Does wood with bark that has been treated remain suitable for colonisation by pests and pathogens?

If so, what does this mean for risks of ISPM15 compliant wood with bark moving in international trade?

Question 1 has been investigated through IFQRG and the results from the UK studies are summarised below.

RESULTS FROM UK STUDIES ON ISPM15 TREATMENT AND RESIDUAL BARK

In the UK, there was significant attack by bark and wood boring beetles when heat treated pine wood with bark present was placed in the forest during the spring period when several species of bark and wood boring beetles were active. The experiments included roundwood as well as sawn wood, laid out in randomised designs to provide a range of combinations of heat treated/not treated and bark/no bark. The results are summarised in Table 1.

Table 1. Emergence of bark beetle species, following successful breeding, from pine wood, with or without Heat Treatment and bark, exposed to natural attack of xylophagous insects in Alice Holt Forest, UK

Bark beetle species	Sawn wood			Logs	
	control	heat treated	kilned	control	heat treated
Tomicus piniperda	595	22	–	13204	743
Hylurgops palliatus	12	15	5	2	3656
Orthotomicus laricis	–	309	–	21	518
Hylastes ater	25	21	3	49	114
Hylastes sp.	14	6	1	81	101
Ips sexdentatus	–	2	–	78	72
TOTALS	646	375	9	13435	5204

These data provide a clear answer to Question 1:

Most species of endemic pine bark beetle demonstrate an ability to utilise heat treated timber with residual bark.

There was some evidence for re-infestation within the emergence cages; bark on logs had remained suitable for further attack several weeks after heat treatment was carried out.

Overall, heat treatment of bark on freshly felled logs and cut wood does not make it unsuitable for successful colonisation and emergence by a range of bark beetle species.

Question 2: Treated wood with bark supports bark beetles. What does this mean for risks of ISPM15 compliant wood moving in international trade?

The question of whether presence of bark poses a significant threat even after active ISPM15 treatment is currently under debate and, as part of this debate, the numbers of bark and wood boring beetles that would constitute a threat to a receiving country is being considered. It is evident that the numbers of beetles that could complete development to adult emergence on any residual bark on ISPM15 compliant wood may constitute founder populations of the pest in a new country. However, there is no simple way of determining the size of a viable founder population and, therefore, how much bark would be needed to support such a population. Analysis of interceptions and, more importantly, successful establishment of bark beetles associated with international trade suggests that bark beetles with different strategies and damaging potentials have very different establishment requirements in new locations.

Analysis of interception data from several countries has been carried out using internet resources and other data such as those provided by Brockerhoff *et al.* (2003, 2006), Haack (2001, 2006) and personal communication with scientists and quarantine regulatory staff in many countries.

While inevitably being an underestimate of the range of bark and wood boring insects moving and/or establishing in new locations, the analysis provides some interesting insights into the likelihood of establishment of this potentially damaging group of insects. The results of interceptions reported by a number of countries are presented, as proof of initial transfer along the ‘wood’ pathway, in Table 2.

The categorisation into different strategies of attack ranges from ambrosia beetles through secondary pests that can only attack weakened or dead trees to primary tree killers; the latter category is divided into aggressive and non-aggressive (Paine *et al.* 1997). What is apparent from these lists is that, irrespective of the attack strategies of the pests, they are able to survive transit in wood and emerge in the destination country. The obvious question is, therefore, can they establish successfully in the new location? Further analysis of literature and interception information provides some insight into the numbers of beetle species that have established in new locations. The results are summarised in Table 3, which also lists the numbers of established species in relation to the numbers intercepted in trade.

As might be expected from their attack strategies, there is ample evidence of establishment of ambrosia and secondary bark beetles that tend to attack dying or weakened trees, sometimes supported by fungal associates. What is, perhaps, more surprising is the reduced or nil success rate of the primary tree killers, particularly those

Table 2. Interceptions of bark and wood boring beetles in Australia, Canada, EU, New Zealand, South Africa and USA

Nature of pest	Genera
Ambrosia beetles. Secondary wood borers in weakened, dying or recently dead trees	*Arixyleborus* *Cryptoxyleborus* *Euwallacea* *Gnathotrichus* *Trypodendron* *Xyleborinus* *Xyleborus* *Xylosandrus* *Xyloterinus* Platypodidae
Xylophagous (wood boring) bark and longhorn beetles. Mainly in weakened, dying or recently dead trees but some attack healthy trees	*Cerambycidae* *Anoplophora* *Arhopalus* *Callidielum* *Ceresium* *Hylotrupes* *Hypothenemus* *Lagocheirus* *Megopis* *Monochamus* *Phoracantha* *Phymatodes* *Psacothea* *Rhagium* *Saperda* *Stephanoderes* *Tetropium* *Trichoferus* *Xylotrechus* *Scolytinae* *Dryocoetes*
Secondary bark feeders. Weakened, dying or recently dead trees	*Carphoborus* *Cryphalus* *Crypturgus* *Dryocoetes* *Dryocoetinus* *Hylastes* *HylesinusHylurgopinus* *Hylurgops* *Hylurgus* *Hypothenemus* *Ips (some spp.)* *Lesperisinus* *Monarthrum* *Orthotomicus* *Phloeosinus* *Pityogenes* *Polygraphus* *Pseudohylesinus* *Pteleobius* *Scolytus (some spp.)Stephanoderes* *Tomicus*
Primary but non-aggressive tree killers	*Dendroctonus valens* *Ips acuminatus* *Dryocoetes confusus* *Ips cembrae* *Ips grandicollis* *Ips pini* *Ips plastographus* *Ips subelongatus* *Scolytus ratzeburgi* *Scolytus ventralis* *Scolytus subelongatus*
Primary aggressive tree killers	*Dendroctonus brevicomis* *D. frontalis* *D. ponderosae* *D. pseudotsugae* *D. rufipennis* *Ips amitinus* *Ips typographus*

classified as aggressive. Thus, some extremely damaging bark beetles in their countries of origin do not appear to have established successfully outside their home ranges. This suggests that the dynamics of founder population establishment may provide insights into the quantitative basis of pest risk analysis for insects associated with wood and,

Table 3. Proof of pathway for establishment; the numbers of bark beetle genera/species that have established successfully in new locations as a result of transfer in international trade

Nature of pest	Genera		
Ambrosia beetles. Secondary wood borers in weakened, dying or recently dead trees	*Dryoxylon*	*Euwallacea*	*Gnathotrichus*
	Trypodendron	*Xyleborus*	*Xyleborinus*
	Xylosandrus	*Xyloterinus*	
	Platypodidae	3 species	11/11+
Xylophagous (wood boring) bark and longhorn beetles. Mainly in weakened, dying or recently dead trees but some attack healthy trees	Cerambycidae		
	Anoplophora	*Callidielum*	*Hylotrupes*
	Phoracantha	*Phymatodes*	*Rhagium*
	Saperda	*Tetropium*	8/19
Secondary bark feeders Weakened, dying or recently dead trees	*Cryphalus*	*Crypturgus*	*Hylastes*
	Hylesinus	*Hylurgops*	*Hylurgus*
	Hypothenemus	*Ips* (some spp.)	
	Lesperisinus	*Orthotomicus*	*Phloeosinus*
	Pityogenes	*Polygraphus*	*Pteleobius*
	Scolytus (some spp.)	*Stephanoderes*	*Tomicus*
			17+/23+
Primary but non-aggressive tree killers	*Dendroctonus micans*		*Dendroctonus valens*
	Ips acuminatus	*Ips cembrae*	*Ips grandicollis*
			4/11
Primary aggressive tree killers	None recorded		0/7

therefore, the significance of bark as a carrier of some of the most dangerous wood-killing beetles.

Do non-aggressive and aggressive xylophagous pest species have different establishment strategies and founder population sizes?

The quantitative basis for founder populations of bark and wood boring beetles has not been established, particularly for the aggressive tree killers, although there are some indications that relatively large aggregated populations are required. For example, Berryman et al (1985) determined that the threshold for successful attacks by mountain pine beetle, *Dendroctonus ponderosae*, was 58 beetles m^{-2} for weakened trees and 119 beetles m^{-2} for the most vigorous trees. Thus, even on weakened trees, this represents a large pioneer population of bark beetles. Clearly, the numbers that could result in successful attack will vary with beetle species and their attack strategies so that, at the other extreme, a single female of great spruce bark beetle, *Dendroctonus micans*, is capable of colonising a living tree successfully (Evans 2001).

Founder population sizes are of particular relevance to the debate on presence of bark on ISPM15 compliant packaging wood and this has been a primary focus for IFQRG. Following the IFQRG meeting in December 2005, in which data were analysed from studies carried out in Canada, Germany, UK and USA as well as survey data of imported wood done by Australia and the EU (http://www.forestry-quarantine.org/docs-Dec1-05/IFQRG_Statement_ISPM15Dec05.pdf), the following conclusions were reached:

Experiments conducted in 2004 and 2005 were designed to ensure that the treated wood used in the experiments would be exposed to pest attack. Material used in the research was freshly cut, green wood. These experiments clearly demonstrate that wood with individual pieces of bark as little as 25 cm^2 and treated under ISPM-15 requirements could be infested by pests that are of phytosanitary concern. Many of these same pests were not found to infest wood that had all bark removed.

Information collected by Australia indicated that 0.5% of ISPM-15 marked material inspected at the point of entry was infested by organisms of phytosanitary concern. This analysis suggested that material with 10 cm^2 or more bark had a higher level of infestation than material with small or no amounts of bark. The European Union and Canada have also reported similar levels of interceptions to Australia on marked wood. Due to uncertainty in this information of whether or not infestation occurred post treatment, a causal relationship cannot be determined between the presence of bark and infestation of ISPM-15 compliant material.

Further studies have been carried out by Robert Haack and colleagues (this volume) and indicated that approximately 0.11% of ISPM15 marked wood entering the USA had bark with live insects present. However, the authors were not able to determine whether this was infestation after treatment or failure of treatment.

Irrespective of the precise reasons for the presence of live insects on ISPM15 compliant packaging wood, further analysis is required to determine the significance of insect presence in relation to potential founder populations of pests. The studies carried out in the UK indicated that successful breeding of bark beetles was possible in sawn wood and that an average of around 1 beetle per 36 cm^2 of bark emerged in the studies, although some pieces were much more heavily attacked. Similar results were obtained by Thomas Schroeder in Germany (http://www.forestry-quarantine.org/analysis%20 Bark-ISPM15.pdf), with results suggesting that successful breeding of bark beetles could take place in 48 cm^2 bark pieces.

Taking the evidence as a whole, the factors that are likely to affect founder population size and likelihood of establishment include:

Survival in transit: there is ample evidence of successful transit and interception on arrival (Table 2). Specific surveys for the presence of live insects on ISPM15 compliant wood are now being carried out.

Proximity to suitable hosts after arrival: this is a probability event involving numbers of emerging adults, dilution of the dispersing pioneer populations, sex ratio/mate finding and distance to suitable host tree species. Table 3 indicates that non-aggressive xylophagous species have established successfully. However, there is no evidence for establishment of aggressive species, suggesting that there may be more stringent requirements for successful founder population performance.

Thresholds for attack on weakened/healthy trees: non-aggressive species are successful. Presumably, aggressive species could also breed on weakened trees, but this does not appear to be the case. Is the emergent population too small to continue and becomes extinct because of Allee effects (Allee *et al.* 1949, Tobin *et al.* 2007) or to random stochastic effects or a combination.

Synchrony: is the timing of initial emergence of pioneer adults synchronised with availability of hosts and suitable temperature and day length requirements?

Do aggressive species need ‘mass attack’, even on weakened trees?: there is evidence that founder population size is greater, even on weakened trees (Berryman *et al.* 1985).

CONCLUSIONS

There is an increasing body of evidence that wood that has been treated to ISPM15 standards can harbour bark and wood-boring beetles, with a minimum size of bark piece of around 25-35 cm^2. The shape of the bark piece is important and elongated pieces, e.g. along the edges of boards, are less able to support successful breeding. Even small populations of non-aggressive bark beetles may represent viable founder populations, e.g. *Dendroctonus micans* has incestuous mating and a single emergent female can successfully attack a healthy tree. By contrast, it appears that much larger founder populations are required for successful establishment of aggressive bark beetles and there may, therefore, be a differential risk in pathway analysis of this group of pests. However, detailed quantitative studies are required to determine the scale of this risk and, in any event, some apparently innocuous species can be considerably more damaging in new ecosystems, suggesting that presence of bark may pose a genuine risk even in ISPM15 compliant wood.

Acknowledgements. *The UK bark studies were carried out in collaboration with Martin Jukes and Christine Tilbury of Forest Research and I would like to thank them for their excellent work on this important subject.*

References

Allee W. C., Emerson A. E., Park O., Park T., Schmidt K. P. 1949. Principles of Animal Ecology. W. B. Saunders, Philadelphia, PA.

Berryman A. A., Dennis B., Raffa K. F. N. C. 1985. Evolution of optimal group attack, with particular reference to bark beetles (Coleoptera: Scolytidae). Ecology, 66: 898-903.

Brockerhoff E. G., Knížek M., Bain J. 2003. Checklist of indigenous and adventive bark and ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) of New Zealand and interceptions of exotic species (1952–2000). N.Z. Entomol., 26: 29-44.

Brockerhoff E. G., Bain J., Kimberley M., Knížek M. 2006. Interception frequency of exotic bark and ambrosia beetles (Coleoptera: Scolytinae) and relationship with establishment in New Zealand and worldwide. Can. J. For. Res., 36: 289-298.

Evans H. F. 2001. Management of pest threats. [In:] The Forests Handbook. Volume 2. Applying Forest Sceince for Sustainable Management (ed. Evans J.), pp. 172-201. Blackwell, Oxford.

Haack R. A. 2001. Intercepted Scolytidae (Coleoptera) at U.S. ports of entry: 1985–2000. Integr. Pest Manage. Rev., 6: 253-282.

Haack R. A. 2006. Exotic bark- and wood-boring Coleoptera in the United States: recent establishments and interceptions. Can. J. For. Res., 36: 269-288.

Liebhold A. M., Macdonald W. L., Bergdahl D., Mastro V. C. 1995. Invasion by exotic forest pests: a threat to forest ecosystems. Forest Science Monographs, 30: 1-49.

Paine T. D., Raffa K. F., Harrington T. C. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annu. Rev. Entomol., 42:179–206.

Tobin P. C., Whitmire S. L., Johnson D. M., Bjørnstad O. M., Liebhold A. M. 2007. Invasion speed is affected by geographical variation in the strength of Allee effects. Ecology Letters, 10: 36–43.

THE IDEA OF FOREST PROMOTIONAL COMPLEX „PUSZCZA KOZIENICKA” WITH SPECIAL REGARDS TO TOURISM STRATEGY

Katarzyna Supernak, Piotr Kacprzak, Marek Krzemiński

City Hall Radom, Poland, e-mail: kaska_supernak@wp.pl

Tourism has recently become a rapidly developing industry unavoidably linked to the environment. Nature responds to this sort of activity in diversified ways. So far in Europe the pressure that the tourism industry exerted on afforested areas has been relatively low as compared to the other environments such as sea coasts or lake districts. It may be said, however, that this pressure, especially on protected areas, will grow significantly in the future, as the requirements of the people seeking recreation concentrate on “green” or “countryside” tourism. Many European governments regard tourism as a matter of precedence looking forward to satisfactory profits from investment in the industry. Tourism is also considered a factor catalyzing local economic activities. Forests are being perceived as having great potential for various forms of recreation, for instance, as tourism connected with environmental education. The growing number of touris builds up an increasing pressure for the improvement of infrastructure in constantly developing tourism capacities, mainly in economically emerging regions and – for this reason – the most precious in terms of abundance of natural resources.

Tourism activity is not equal in the all afforested areas. There are many locations, environments and species (animal and plants) that may be deeply affected by various detrimental impacts of tourism. These are pollution, erosion, collecting specimens and breaking the natural silence. The most drastic interference in the long term is the development of tourism facilities such as villages, roads, ski slopes etc. Such activities often place valuable environments at risk and may result in their complete destruction. The pressure of this impact may be reduced by cautious and all – inclusive planning, controlling the direction of tourism development and its monitoring. The evaluation of the sensitivity of forests to damage caused by tourism may be very helpful in determining the capacity for recreation in various types of woods. An increasing awareness of recognizable environmental hazard closely connected with the negative effects of industrial civilization has stimulated the interest of the people considerably over the recent years. People feel that they need a closer contact with Nature – a contact comprehended not only as an inclination to spend their leisure time in the woods – but also as a need of cognition and understanding.

Fig. 1 The Forest Promotional Complexes, FPC

The Forest Promotional Complexes show in Fig. 1 are afforested locations where multi – functional management of forest economy based upon ecological foundations is promoted and implemented. The forest promotional complexes were defined as the larger and compact afforested areas composed of one or more forest inspectorates set up in order to promote the pro-environmental policy of the State. FPCs include usually the entire inspectorate, rarely the separate parts of so called districts. FPCs are therefore functional units having no separate administration which is carried out by inspectorates supervised by Regional Management of the State Forests (Krzemiński 2003). The main reason for which the FCPs have been established was to separate and protect the forests of exceptional value in terms of ecology, education and social perception.

The Kozienicka Forest was one of the first FPCs set up in Poland (Fig. 2). It was established by the ordinance the general director of The State Forests dated December 19th 1994 but its final limits were corrected by the second ordinance #25 by the same authority on August 11th 1995.

In respect to its scenery and natural qualities a natural scenic area was acknowledged in 1983 and has the status of the Forest Promotional Complex since 1994.

The Kozienicka FPC is composed of 15 reservations in three inspectorates:

- Inspectorate Kozienice (districts Kozienice, Pionki, Zagożdżon),
- District Jedlnia of Inspectorate Radom,
- Districts Garbatka and Zwolen of Inspectorate Zwolen.

All of them belong to the Inspectorate of Radom.

The management of the forest economy in FPC is conducted in accordance with principles respecting nature protection and providing:

- protection of biological diversity,
- multi – functional aspects of the Forest,
- sustainable development (three functions) as shown in Fig. 3.

The main prerequisite conditions to establish The Kozienicka FPC are as follows:s

- to keep the wealth of natural resources,
- cultural values originating from the forest economy,

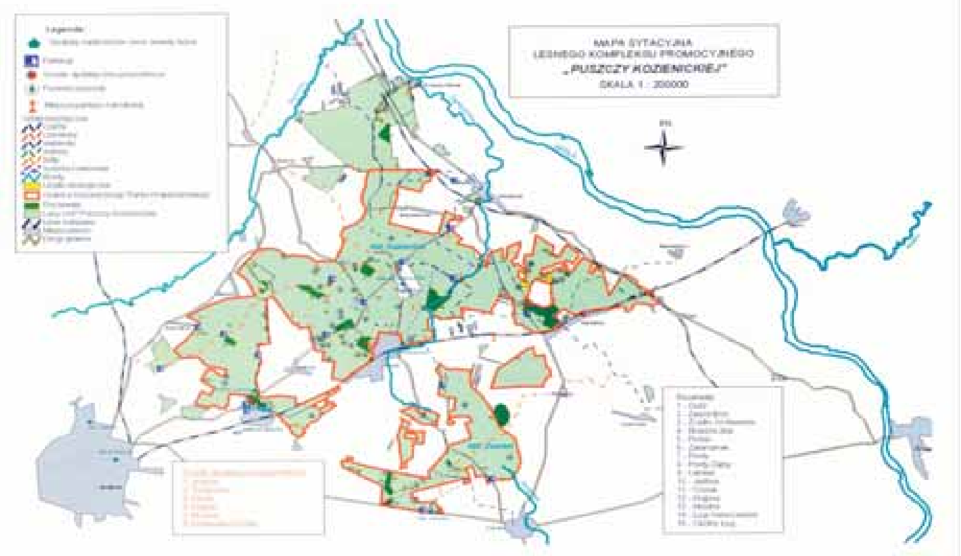


Fig. 2. The map of Kozienicka Forest and the Forest Promotional Complex

– historic traditions,

– and to utilise the best outputs of science and good forest practices.

The interest of the state government, and non – governmental organizations is to sustain forest functions other than utility and production of wood and an environmental hazard to forest ecosystem (Pajek 1997). The most valuable qualities of the Kozienicka FPC are (Bielecki 1997):

– the abundance of forest habitats (from fresh coniferous forest to marshy meadows),

– the large share of fertile forest stations,

– the abundance of the plant kingdom at diversified species, age and height level structure,

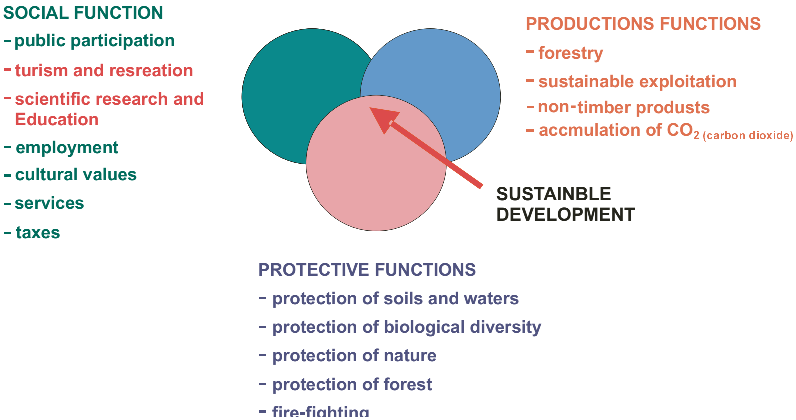


Fig. 3 The functions of sustainable development in FPC

– the large areas of natural tree stands,

– the basic seed collecting area,

– the moderate climate and abundance of animal kingdom,

– the significant number of natural reserve (area over 100 ha) and monuments of nature.

The Kozienicka FPC is a unique abundance of plant and animal kingdoms. There are nearly 600 species of vascular plants, 200 species of lichen and 94 species of moss existing in 20 different forest communities. The most meaningful are fresh coniferous forest, fresh mixed coniferous forest, fresh mixed broadleaved forest, oak-hornbeam forest, bright oak forests, alder swamp forest and boggy mixed pine forests, where 36 species of trees can be found. The most common are pine, common oak, sessile oak, black alder, silver and hair birch, fir, great maple and common beech. Among the shrubs there are 33 species where the most common are hazel, black dogwood and juniper. On the level of forest vegetation cover, one can find more than 40 protected species (among which are daphne, common ivy, stagshorn and interrupted clubmoss).

Facing the growing needs in this respect the foresters make available to the people the most interesting forest locations which are not only a tourist attraction but also a living source of environmental education. This target is being achieved through

Table 1. The objects exploited for the purpose of tourism and recreation

Objects	FPC Kozienicka Forest	
	Number of facilities	Length in km
Marked tourist routes	9	180
Biking routes	–	235*
Forest parking lots	29	–
Farm tourism	20	–
Water reservoirs	4	–

*including projected biking routes

Table 2. The objects for educational purposes

Objects	FPC Kozienicka Forest	Regional Managment of the State Forest
Educational paths	9	29
Education center	1	1
Didactic spots	3	10
„Green classes”	3	15
Educational spots	5	25

building parking lots, camping places, hiking and biking trails, viewpoints and didactic paths (shown in Table 1 and 2).

References

Bielecki J. 1997: Cele i zadania Leœnego Kompleksu Promocyjnego “Lasy Puszczy Kozienickiej”. [In:] Edukacja œrodowiskowa. Agenda 21 – realizacja zadañ edukacyjnych, IBL, Warszawa.
Kozienicki Park Krajobrazowy. Mapa przyrodniczo – krajobrazowa 1:75000, 2002. Zarz¹d Kozienickiego Parku Krajobrazowego. Pionki.
Krzemiñski M. 2003: Leœne kompleksy promocyjne elementem ekorozwoju. PWSOŒ.
Operat Urz¹dzeniowy Nadleœnictwa Radom na lata 1996–2005.
Pajek T. 1997: Leœny Kompleks Promocyjny „Lasy Puszczy Kozienickiej”.[In:] Lasy Puszczy Kozienickiej (ed. R. Zielony). Wydawnictwo SGGW, Warszawa.
Szujecki A. 2000: Leœne Kompleksy Promocyjne – egzemplifikacja proekologicznej polityki leœnej Pañstwa. [In:] Postêpy techniki w leœnictwie. Stowarzyszenie In¿ynierów i Techników Leœnictwa. Warszawa.
Zarz¹dzenie Dyrektora Generalnego Lasów Pañstwowych z dnia 19 grudnia 1994 w sprawie leœnych kompleksów promocyjnych.
www.lasypanstwowe.gov.pl
www.kozienickipk.com2
www.radom.lasy.gov.pl

PART II

ABSTRACTS

International forest quarantine activitie

Eric Allen

Natural Resources Canada, Canadian Forest Service, Victoria, British Columbia, Canada

Protecting the world's forests from alien invasive pest species requires the coordinated efforts of the scientific, industrial and regulatory communities. In recent years, a number of international groups have been formed to address critical forest quarantine issues. The International Forest Quarantine Research Group (IFQRG) has about 60 members representing over 20 countries. This group is an independent body that provides advice to policy makers and international regulators. It provides scientific analysis and reviews of global phytosanitary issues, undertakes collaborative scientific research and provides a forum for the discussion and clarification of key issues related to the phytosanitary implications of global trade. The Technical Panel on Forest Quarantine (TPFQ) is a formal panel of experts associated with the International Plant Protection Convention. This group helps develop international standards (such as ISPM No. 15) for adoption by the global phytosanitary community. It is important that the IUFRO WG "Invasive Alien Species in International Trade" work together with these groups to bring the best scientific information forward to minimize the movement of global forest pests.

Recent dieback of *Fraxinus excelsior* in Sweden

Remigijus Bakis, Pia Barklund, Katarina Ihrmark, Jan Stenlid, Rimvydas Vasiliauskas

Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences,
Uppsala, Sweden

In the summer 2004 decline and death of common ash (*Fraxinus excelsior* L.) were reported widely in Sweden and in 2005 it increased further. Detailed investigations of the disease symptoms showed leaf death, shoot dieback, branch and stem bark necrosis ultimately leading to tree death generally over the whole area where ash is growing naturally. Ash decline was known only in a few places in 2002, indicating a rapid and recent increase of an alarming emerging disease. Pilot investigations have revealed one fungal species, which has been consistently isolated from dying tissues. Re-inoculations into healthy shoots have reproduced the disease symptoms. The causative pathogen is still not fully described and the infection biology and environmental conditions that promote the disease development are unknown.

Environmental and economic effects of invasive forest pests moved in nursery stock

Kerry O. Britton, Joseph Lewis

USDA Forest Service, Forest Health Protection, 1601 N. Kent Street,
RPC-7 Arlington, VA 22209, USA

Many devastating forest diseases and insect pests have been introduced to the United States via nursery stock. Beech bark disease, for example, arrived in Halifax Nova Scotia in the late 1800s. Radial growth reductions of 20-40% have been documented in affected beech stands. Extensive mortality results in gaps which are colonized either by root sprouts of beech or by other species, depending on stand history and other disturbances. Port Orford Cedar root disease, Chestnut Blight, Hemlock Wooly Adelgid, White Pine Blister Rust, Balsam Wooly Adelgid, larch casebearer, and red pine scale are other examples of invasive pests imported on nursery stock which will be discussed. It is important to evaluate the economic and ecological costs of invasive species, but it is also extremely difficult. Available estimates generally rely on the direct costs of control programs, for which empirical data is readily available, or on lost growth or potential losses of merchantable timber, for which empirical data is usually lacking, and expert judgment must be relied upon. This process overestimates losses if the resources in peril would not be harvested for other reasons. Estimates of ecological losses require placing some monetary value on the ecosystem services provided by the species in question, and such estimates are extremely rare. Since undocumented estimates damage credibility, assumptions made in developing estimates must be transparent. While regulatory choices should be informed by balancing the costs and benefits of potential management activities, usually the true costs of an invasive species incursion are not known at the time when decisions regarding desirability of implementing a rapid response is needed.

Pathways and trade policy: the dynamics of invasive alien species in the forestry sector

Stas Burgiel

Global Invasive Species Initiative, The Nature Conservancy 4245 North Fairfax Dr.,
Suite 100, Arlington, VA 22203 USA

This presentation would provide the background and policy context for pathways through which invasive alien species move, as well as for trade in forest products. Starting with a brief overview of the pathways concept and work by the Pathways Work Team under the U.S. National Invasive Species Council, the talk would then discuss pathways affecting the forestry sector, including nursery stock, softwood packaging material, raw lumber and related vehicular transport. The international policy context would then be addressed, including relevant work under the Convention on Biological Diversity, the International Plant Protection Convention and the World Trade Organization (WTO). This would lead into a broader discussion about trade policy, including relevant WTO rules and agreements, as well as the allowances and limitations for regulating pathways for introduction as opposed to individual invasive species. Other items for discussion include relevant aspects of the WTO's current Doha Development Round of negotiations, such as an initiative to liberalize trade in forest products, as well as bilateral and regional free trade agreements. The presentation will close by highlighting general recommendations, existing research and policy gaps, and potential next steps.

Species composition of *Cameraria ohridella* Deschka&Dimic (Lep.,Gracillariidae) parasitoids from Poland

Katarzyna Celmer-Warda[1], Cezary Bystrowski[2], Grzegorz Tarwacki[2]

[1] University of Agricultural, department of Applied Entomology, ul. Nowoursynowska 161,
Warsaw, Poland
[2] Forest Research Institute, Department of Forest Protection, Sękocin Stary, ul. Braci Leśnej 3,
05-090 Raszyn, Poland

Parasitic Hymenoptera from the suprafamily Ichneumonoidea attacking the horse chestnut leafminer *Cameraria ohridella* Deschka&Dimic (Lepidoptera: Gracillariidae) were studied in central Poland. During 2005-2006 infested fall leaves of *Aesculus hippocastanum* L. were collected in 8 diversified stands in last days of October, before frozen and in Spring (in first days of April).

A total of 14 species of parasitoids were found on *C. ohridella* leafminer. They belong to two suprafamilies of Hymenoptera, Chalcidoidea and Ichneumonidea. The most abundant species were *Minotetrastichus frontalis*, *Pnigalio agraules*, and *Pteromalus semotus*. They dominated in every sample sometimes up to level of 90%.

Another species of Chalcidoidea were collected regulary : *Closterocerus trifasciatus, Cirrospilus pictus, Cirrospilus talitzkii* and species of Ichneumonidea : *Scambus alternans*, *Itoplectis sp.*
Percent of parasitism generally depends from the locality. The highest parasitism (up to 70%) was observed in sunny places characterized by highest temperature during vegetation season. Samples collected in dense stands (e.g. chestnut trees in Modrzewina Rez.) were characterized by lower level of parasitism. The lowest parasitism (about 15%) level was observed in locality with removed fall leaves.

The application of phytosanitary systems in Africa- the sadc experience

Clement Chilima, George Phiri

Forestry Research Institute of Malawi, P.O Box 270, Zomba, Malawi

The paper reviews the application of phytosanitary systems in Africa with examples from countries in the Southern African region. We describe the permit systems for import and export of forest products in the SADC region and question the extent to which these apply to the international agreements on Sanitary and Phytosanitary (SPS) under WTO. We identify many weaknesses including lack of efficient inspections, associated with inadequate human and material capacity and lack of risk assessment on prospective importations of forest products and materials. We describe the current processes in Africa where through networking, harmonised approaches to invasive species that invaded the Africa are being developed.

Q-TRAP^{TM}: detection of bioinvasive insects

Darek Czokajlo[1], Philipp Kirsch[1], Aubrey Moore[2]

[1] IPM Development Company, Portland, OR, USA
[2] University of Guam, Guam, USA

Invasive alien species are non-native organisms that cause, or have the potential to cause, harm to the environment, economy or human health. Every new introduction causes not only economical damage through destruction of native plants and associated industries but also by potential displacement of native species thus destabilizing an ecological balance. Biotic invasions can occur when organisms are transported to new, often distant, ranges where their descendants proliferate, spread and persist. Over 2000 exotic insect species are now established in the US, 20% of which feed on trees and shrubs. National quarantine agencies are hard pressed to keep up with burgeoning volumes of global trade. Between 1985-2000, 577,829 insects were intercepted in the United States ports, 7,890 (1.4%) were associated with wood, timber or wooden packing materials.

Within the context of rapidly increasing trade, risks of biological invasions are also increasing. Concern over the potential for species introductions to be used strategically as instruments of bio-terrorism or bio-warfare appears to be well founded. Growth in the volume and complexity of international trade, combined with the liberalization of regulatory regimes to encourage trade, has at once increased the frequency of introductions along existing pathways, the number of new pathways, and the ease at which potentially invasive species can move along those pathways. The deregulation of national and international markets has reduced both the barriers to trade and the surveillance of trade, thereby increasing the risks of invasions.

Our group has recently optimized detection systems for a wide range of invasive insects of interest to global quarantine agencies. Starting with optimization of Pine Shoot Beetle traps in North American and Europe, our research and development has expanded to include *Sirex* wood wasps in Australia and North America; Old House Borer in Western Australia; and port detection of *Ips* bark beetles and *Monochamus* borers for port detection. An increase in the availability, timeliness, accuracy and

transparency of pest information is expected to lead to more confidence in the quarantine integrity of cargo and increased detections of invading species, resulting in greater efficiencies in cargo transportation, while reducing risks associated with inadvertent introduction of invasive species.

Early and rapid detection of forest invasive species

Mary Ellen Dix, Robert Rabaglia

US Department of Agriculture, Forest Service, Research and Development VMPR 4th floor RPC,
Kent Street, Arlington, Virginia USA

Early detection of a newly arrived invasive species is key to successful prevention and eradication efforts. The United States Forest Service and our partners are developing and providing tools for the detection, monitoring, enforcement and eradication to federal and state agencies so that they can eliminate invasive species populations and prevent economic loss and ecological damage to the nation’s forests and rangelands. This talk will examine the tools already available and potential new tools for detection of an invasive species and identify crucial knowledge and tools gaps.

Science and policy issues of invasive alien species – challenges and opportunities

Jacques Gagnon

Director, Science Policy, Natural Resources Canada – Canadian Forest Service, 580 Booth Street,
7th Floor, Ottawa, Ontario K1A 0E4, Canada

International trade, exponentially increasing everyday, is the major entry point for invasive alien species (IAS), and ultimately increasing the irreparable damages to biodiversity. Loss of biodiversity not only influences on economic and social issues but, more importantly, obstructs the ecological processes, indicating the complexity in addressing the policy issues of invasive alien species.

Science based policies are indispensable to address the issues of invasive alien species, and are linked to several disciplines and extend to multi-jurisdiction. Developing such policy is a challenge in the situation where communication between policy makers and scientists is narrow. However, the process of developing IAS policy creates opportunities to share and interact among scientists and policy makers from different fields. In the end, both science and policy needs benefit from integrated efforts.

Ceratocystis albifundus, an African fungus threatening flora internationally

Ronald N. Heath, Jolanda Roux, Brenda D. Wingfield, Michael J. Wingfield

Department of Genetics, Tree Protection Co-operative Programme (TPCP),
DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB),
Forestry and Agricultural Biotechnology Institute (FABI),
University of Pretoria, Pretoria, South Africa

The ascomycetous fungus originally identified as *Ceratocystis fimbriata* and now known as *Ceratocystis albifundus* was first collected from native *Protea gaguedi* plants in South Africa in 1977. It was, however, the discovery of this fungus causing a serious wilt disease on the non-native and important forest plantation tree, *Acacia mearnsii*, which first led to studies regarding its taxonomy and pathogenicity. *C. albifundus* has now been found infecting wounds on 14 native African tree species. These species represent various families including Anacardiaceae, Caesalpiniaceae, Combretaceae,

Fabaceae, Mimosaceae, Ochnaceae, Proteaceae and Rosaceae and records of infections on these trees support molecular genetic evidence that the pathogen is native to Africa.
Ceratocystis albifundus is considered as the most serious pathogen of *A. mearnsii* trees grown in plantations in South Africa. Symptoms associated with infection of these trees include vascular staining and streaking, cankers, wilting and tree death. Infection usually occurs through wounds caused by corrective pruning practices and hail damage. Recent studies have indicated that *C. albifundus* is vectored by at least four insect species. These include three species of *Carpophilus* and one species of *Brachypeplus,* all belonging to the sap feeding Nitidulidae. *Carpophilus* spp. have a cosmopolitan distribution with a wide host range including crops such as tomatoes, stone fruits, apples and service-berries. These insects, together with infected plant material could easily spread *C. albifundus* to continents other than South Africa. There are numerous examples where insects have vectored pathogens across international borders and there is good evidence that the closely related wilt and canker pathogen *C. fimbriata* has spread between continents. Introduction of *C. albifundus* into new areas where related species in the tree families listed as it's hosts in Africa occur, could lead to the devastation of native vegetation and even extinction of genera. *C. albifundus* is a highly virulent pathogen of *Acacia mearnsii* with a wide host range and it should be considered as one of the southern hemisphere's more threatening potential alien invasive pathogens.

Invasive forest insect species in Poland

Tomasz Jabłoński, Jacek Hilszczański, Andrzej Kolk, Sławomir Ślusarski, Lidia Sukovata

Forest Research Institute, Forest Protection Department, Sękocin Stary, ul. Braci Leśnej 3,
05-090 Raszyn, Poland

The issue of invasive forest species introduced to Poland form other countries related is mainly to import of unprocessed (unbarked) timber or other wood products like wooden packages. Those materials were in the past imported to Poland infested by bark and wood boring insects including beetles of the families: Lyctidae, Bostrychidae, Curculionidae, Platypodidae Cerambycidae and wood wasps Siricidae. Potentially dangerous could be Asiatic species recorded form our country imported with wooden materials and are known to infest living deciduous and coniferous species i.e. Xyleborus perforans (Wollanston), Platypus cavus (Strohmeyer), Platypus solidus (Walker), Anoplophora glabripennis (Motschulsky). Of minor importance as pests are wood borers imported with processed materials like wooden boxes or wicker baskets, which infest dead wood i.e. Sinoxylon anale (Lesne), Lyctus brunneus (Steph.), Gracilia minuta (F.) and Nathhrius brevipennis (Muls.).

Estimate of air drying time for ispm-15 compliant wood packing material in international trade

Barbara Illman[1], Xiping Wang[2]

[1]USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin USA
[2] Natural Resources Research Institute, University of Minnesota, Duluth, Minnesota USA

Wood packing material (WPM) is a major pathway for the global transport of invasive species during international trade. The Asian longhorned beetle is believed to have been introduced into the United States on wood pallets, one type of WPM. A new International Standards for Phytosanitary Measures (ISPM) has been issued by the International Plant Protection Convention for phytosanitary treatments of wood packing material. Guidelines of the standard, known as ISPM-15, call for treatment of WPM with heat to achieve a minimum wood core temperature of 56 degrees Centigrade for 30 minutes or treatment with methyl bromide to a prescribed specification to kill invasive insects and fungi. Concerns have been raised about the length of time that ISPM-15 compliant wood will remain pest

free and whether reinfestation of pests will occur. This presentation will address the conditions of wood favorable for infestation of WPM during international trade. A significant factor that affects infestation is the moisture content of wood. When the moisture content falls below 20%, fungi and many insects do not grow, with the exception of termites and certain drywood borers (*Lyctidae*) that are usually not of high risk to attack forests. Wood dries as it ages, but drying times vary with temperature and relative humidity. Published data of the amount of time for air drying of stacked lumber in specific geographic locations in North America were converted to minimum and/or average temperatures and humidity for the regions and used to develop estimates of air-drying time for wood species and sizes used in WPM. A published multiple regression analysis method may be useful in predicting WPM air-drying time from published and/or new experimental data. The goal of this work is to provide information that will be helpful to assess the quarantine risk of WPM infestation after phytosanitary treatments, to find knowledge gaps about wood viability as a host to pests of quarantine interest that might be needed for the risk assessment, and to provide information about wood moisture of WPM that may help with the risk assessment of mold growth on WPM.

What is the role of biodiversity in invasive ecology?

Nod Kay

Forest Biosecurity & Protection, Ensis, New Zealand

In 1958 Elton proposed that community resistance to invasion increased in proportion to its species' biodiversity – the number of species in that community. Oceanic islands are often quoted as examples of high immigration of alien species to habitats of low, or disharmonic, biodiversity and New Zealand's strict regulatory requirements for all aspects of trade are based on this largely unsubstantiated premise. However, there is ample evidence to support the notion that high biodiversity also facilitates immigration to continents. While New Zealand does have a disharmonic, depauperate biota, typical of oceanic islands, its indigenous forest tree species have not been colonised by continental invertebrates. Conversely a number of indigenous invertebrates have colonised and caused considerable damage to introduced, phylogenetically distinct, continental tree species. Alien invasive species require hosts on which to establish. In studies comparing the palatability of Southern Hemisphere flora to proven invasive defoliators (*Lymantria dispar, Orgyia thyellina, Teia anartoides & Hyphantria cunea*) the New Zealand flora has been found to be less palatable than that of Europe, Australia or South America.

Low-pressure vacuum treatment as a potential alternative to heat treatment or fumigation for killing *Anoplophora glabripennis* in solid-wood packing material

Z. Chen[1], Melody A. Keena[2], M. S. White[1]

[1] Virginia Polytechnic Institute & State University, Department of Wood Science & Forest Products, 1650 Ramble Road, Blacksburg, VA 24060, US
[2] USDA FS, Northeastern Center for Forest Health Research, Northeastern Research Station, 51 Mill Pond Road, Hamden, CT 06514, USA

The potential of using vacuum technology to kill the larvae of the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), in solid-wood packing materials (SWPM) and other wood products was assessed. Survival of larvae exposed directly to different vacuum pressures (10, 20, and 75 mm Hg) and temperatures (20 and 30°C) for different lengths of time was assessed. Some larvae were also placed in wood with different moisture contents

and exposed to a pressure of 20 mm Hg at 20°C to determine the effects of wood moisture content on vacuum lethal time.

Anoplophora glabripennis larvae can be killed by low-pressure vacuum treatment by evaporative removal of body water. Larvae died after loosing as little as 26% total body weight, and all larvae in the trials that lost 40% weight were dead. Larvae were completely desiccated after 60 to 67% weight loss. The rate of larval weight loss was constant for about the first 30 hours at 20 mm Hg vacuum pressure at 20°C. Pupae and eggs were also found to be susceptible to desiccation using low pressure vacuum treatments.

Desiccation rate was negatively correlated with initial larval weight; the larger larvae took twice as long to loose the same percentage of body weight as smaller larvae. The desiccation mortality response line (intercept -23.2 ± 5.3, slope 15.6 ± 3.6, probit mortality vs. log % weight loss) for *A. glabripennis* predicts that 50% of the larvae will be dead after 31.2% weight loss and that virtually all (probit 9) the larvae will be dead after 56.4% weight loss. Desiccation rate significantly increased if either the temperature was raised or the pressure lowered; for example, the larvae lost weight twice as fast at 30°C (6.17%/h) compared to 20°C (3.35%/h).

Larvae placed in the test wood pieces with 21.6% MC lost weight but at a slower rate than if they had been directly exposed to the same vacuum treatment and the desiccation rate averaged only 1.2 % per hour under 20 mm Hg vacuum pressure at 20°C. About 50% of the larvae held in wood under these conditions had lost 40% body moisture after about 20 hours and were dead. Further work to determine the exact treatment conditions for wood material in a commercial facility will be necessary to prepare documentation needed to have this method considered for adoption as part of the International Standard for Phytosanitary Measures (ISPM 15) which is used by the United States and its trading partners.

Potential consequences of introductions of flight-capable female gypsy moths (*Lymantria dispar*) into areas where the females do not fly

Melody A. Keena[1], Phyllis S. Grinberg[2], and William E. Wallner[1]

[1] USDA Forest Service, Northeastern Research Station, Northeastern Center for Forest Health Research, Hamden, Connecticut 06514, USA
[2] USDA Forest Service, Northeastern Research Station, Newtown Square, Pennsylvania 19073, USA

In female gypsy moths (*Lymantria dispar* L.) two biotypes have been identified, both with apparently fully developed wings, that differ in flight capability and associated traits. Multiple introductions into North America of strains of gypsy moth with flight-capable females have occurred prompting eradication programs. The biggest concern over these introductions is that the capacity for female flight in the introduced strains (and possibly in hybrids between them and the existing flightless strain) might increase the potential rate of spread and thus invalidate procedures developed for detection and delimitation of the strain already present in North America.

Individuals from two populations with different dominant biotypes (Russian and North American) were crossed. This hybridization had the following biological consequences. Lower diapause chill requirements of Russian eggs were retained in their hybrids. The hybrids (F_1 and F_2) had similar chill requirements to that of the Russians and significantly less chill than those from North American. Faster larval growth was retained and accentuated with hybridization. The growth of F_1 hybrid larvae was as fast or faster than that of the respective strain of the female parent. This suggests that the presence of hybrids in North America could alter the timing of the presence of various life stages in populations where they are present.

Some female flight capability was retained with hybridization, but the proportion of the population with strong directed flight was reduced. No females from the North Carolina strain attempted to fly and approximately 90% of the females from the Far East Russian strain were capable of strong directed flight. Virtually all of the F_1 hybrids were unable to gain altitude or sustain flight, but about half could glide for a few meters while vigorously flapping their wings. In the F_2 generation, approximately 10-15% of the females were capable of strong directed flight. Female flight capability was found to have a polygenic basis and is likely under the control of at least five genes, of which up to two have one allele that is completely dominant. At two of the loci, one allele is apparently fixed in the Russian population and the other is fixed in the North American population. Heritability of female flight capability was estimated to be 0.60. Variation in wing size, muscle strength, and flight behaviors all appeared to contribute to this flight polymorphism. Thus, continued vigilance to exclude and eradicate introductions of strains capable of female flight in North America is warranted in areas where no females fly. Existing biologically based controls should be effective in controlling or eradicating introduced strains of gypsy moth with females capable of flight.

Stealth invasion: growth of newly founded invading gypsy moth populations

Andrew Liebhold, Patrick Tobin

USDA Forest Service, Northern Research Station, 180 Canfield St., Morgantown, WV 26505 USA
(aliebhold@fs.fed.us , ptobin@fs.fed.us)

A common characteristic observed in many biological invasions is the existence of a lag between the time of arrival by the alien population and the time when established populations are noticed. Recently there have been some important advances in theoretical studies of the population ecology of invasions and these studies shed light on the causes for these lags. In general, these delays are the result of time needed for population growth of populations from founding levels to densities sufficient such that they might be detected. Models indicate that these time lags can be predicted based upon population parameters such as the intrinsic rate of population growth and the diffusion coefficient.

In this paper, we review existing models of the establishment phase of biological invasions and use these models to explore how various life history characteristics influence time lags in population growth during invasions. We further explore these models by applying them to the gypsy moth, *Lymantria dispar*, populations invading N. America. This species has been widely studied and information is therefore available for estimating realistic parameter values. These values are applied to establishment models to predict invasion lags. Results from these models are compared to historical data on gypsy moth establishment and spread. Model predictions are also compared with data describing temporal and spatial lags in wave fronts corresponding to different population levels. Results indicate that, with a few exceptions, models accurately predict lags observed in population growth during the establishment phase.

Finally, we make suggestions on how this information might be used in programs to manage invasions via detection, eradication and containment. Time lags in population growth present problems for management because they allow time for expansion of populations before they are detected and can be eradicated. On the other hand, delays in population growth provide time for planning and delimitation of populations prior to eradication.

Exotic forest insects in Sweden interceptions and establishments

Åke Lindelöw

Swedish University of Agricultural Sciences Department of Entomology, P.O.Box 7044 S-750 07
Uppsala, Sweden

During the last decades millions of m3 of wood material have been imported to Sweden, mainly from Russia and the Baltic states but also from non-European countries. A number of forest insects have been intercepted, but few have been established during these years. No serious forest pest insects have yet been established.

The only serious tree pest insect established is *Cameraria ohridella*, probably hitch-hiking with cars over the bridge from Denmark in 2004.

Many red-listed saproxylic beetle species are recorded in low-quality wood material, which is imported and used as fuel wood to produce energy. Risks for pest species to establish and possible support of rare wood-living species are discussed.

A risk assessment for invasive tree pathogens of alaskan forests

Ewa H. Orlikowska, Paul E. Hennon

Juneau Forest Health Office, USDA Forest Service, State & Private Forestry and Pacific Northwest
Research Station, 2770 Sherwood Lane, Suite 2A, Juneau, AK 99801, USA

We conducted the first risk assessment to determine which tree pathogens would be capable of surviving, spreading, and causing severe disease outbreaks if introduced in Alaskan forests. Currently, Alaska has no significant introduced pathogens. Several pathogens have been introduced, (e.g., black knot of *Prunus* sp., *Dibotryon morbosum*, and white pine blister, *Cronartium* ribicola), but these pathogens do not threaten native tree species. Tree species closely related to native Alaskan trees harbor their own native pathogens in the temperate and boreal forests of Europe and Asia. Our objective was to list and rank major tree disease organisms from the temperate and boreal forests of the world that represent a potential threat to the native tree species in Alaska's forest ecosystems such as *Picea* sp*., Tsuga* sp*., Thuja* sp*., Chamaecyparis* sp*., Populus* sp. and *Betula* sp. We listed similar investigations on invasive tree pathogens, including contacts and publications that were developed in the United States. Next we reviewed foreign literature on tree diseases from Europe and Asia that contained tree genera whose relatives are native to Alaska. Then we ranked the most serious threats to each of the major native tree genera in Alaska. We classified pathogens by type of disease, and for each organism we included details regarding tree genus infected, pathogen name, its synonyms and stages, as well as geographic distribution. We also noted if those foreign tree disease organisms were already present in North America and in Alaska. Our approach assumes that the greatest risk involves the introduction of an invasive pathogenic fungus from the same tree genera that grow in Alaska. We evaluated pathogenic organisms at the species level.
Chrysomyxa abietis, C. ledi var. *rhododendri, C. tsugae-yunnanensis, C. tsugae, Strasseriopsis tsugae, Cistella japonica, Didymascella chamaecyparidis, Lophodermium chamaecyparissi, Melampsora larici-tremulae, Seiridium cardinale* and *Phytophthora lateralis* were considered as the most serious threats to each of the major native tree genera in Alaska. We also listed nine low-ranked pathogens. We discussed the most likely avenues of introduction of the potentially threatening organisms based on natural dissemination and the type of disease caused. The information on the invasive tree pathogens that we collected for this project can contribute to the database "North American Forest Commission Exotic Forest Pest Information System (NAFC-ExFor)". This proactive risk assessment will increase our understanding of which tree pathogens pose the most significant threat to Alaskan forests, and will help in the development of policies and guidelines to minimize the probability of future successful invasions.

The new hybrid-species *Phytophthora alni*: occurance in Bavaria and its devastating interaction with *Alnus glutinosa*

Wolfgang F. Osswald, Christian Clemenz

Section Phytopathology of woody plants, WZW, Technische Universität München,
Am Hochanger 13, 85354 Freising, Germany

In the early nineties an unusual mortality of *Alnus glutinosa* trees was found in southern Britain which was caused by a new *Phytophthora* hybrid with *Phytophthora cambivora* and a still unknown taxon similar to *P. fragariae* as parents *(*Brasier et al., 1999). Recently the pathogen was described as *Phytophthora alni* sp. nov. together with its subspecies *uniformis* and *multiformis* which are found on

A. glutinosa and *A. incana* all over Europe (Brasier et al. (2004)). The pathogen infects fine roots or adventitious roots and grows up into the trunk where it destroys the phloem and cambium tissue. A survey carried out in 2001 and 2002 by the Bavarian State Forestry in riparian and forest alder stands proved that this pathogen was widespread along more than 50% of the river systems and found in about one third of the investigated 3247 forest stands (Jung and Blaschke, 2004).

In order to study the influence of this pathogen on *A. glutinosa,* different physiological parameters of three year old plants either inoculated at the stem basis or infected with soil inoculum were investigated under controlled conditions. Lesion formation on the bark showed a biphasic pattern with moderate growth in spring and strong lesion development end of summer. However, big differences were seen for pathogen growth within the whole tree collective. Infection resulted in strong growth retardation and after two years more than 90% of all infected trees had died. Before death CO_2-uptake rates and transpiration of infected plants were significantly reduced. Consequently minimum leaf water potential values were less negative of infected as compared to control trees, indicating that stomata of inoculated plants were more narrowed. Similar results were also found for about 25 year old riparian *A. glutinosa* trees infected by *P. alni* in the field. In addition starch and sucrose contents of leaves of infected trees were significantly higher as compared to control plants, possibly indicating that destruction of the bark tissue by the pathogen caused a blockage of phloem transport from leaves to roots. Computer tomography studies are ongoing to study and to compare bark and wood destruction by the pathogen of trees harvested in the field with those of three year old infected plants.

Phytophthora **species in Polish forestry and horticulture**

Tomasz Oszako[1], Leszek Orlikowski[2]

[1] Forest Research Institute, Department of Forest Phytopathology, Sękocin Stary,
ul. Braci Leśnej 3, 05-090 Raszyn, Poland
[2] Research Institute of Pomology & Floriculture, Pomologiczna 18,
96-100 Skierniewice, Poland

In 1964 Kozłowska *et al* mentioned *Phytophthora* spp. as potential threats of forest nurseries. After 40 years the severe dieback of alders, caused by *Phytophthora alni*, in forest stands and riparian ecosytems occurred in Poland. The finding of *P. ramorum,* isolated from *Calluna vulgaris* and *Vaccinium vitis-idaea,* indicated on that group of organisms as potential, dangerous pathogen not only in horticulture production but also in forestry. Survey of forest nurseries revealed the occurrence of some *Phytophthora* species and among them *P. citricola* (on *Abies alba*, *Fagus sylvatica, Fraxinus excelsior, Quercus* spp.) *P. cactorum* (on *Alnus glutinosa, Betula verrucosa, F. sylvatica, F. excelsior, Sorbus aucuparia*) and *P. cinnamomi* (on *Pinus sylvestris* and *Quercus* spp.). Till 2005 *P. citricola* was the species the most often detected in diseased plants and soil samples. *P. citrophthora* was isolated from about 40-year-old Norway spruces (*Picea excelsa*) having symptomatic trunk exudates from necrotic bark tissue and rot of fine roots. *P. citricola* and *P. cambivora* were detected in about 80-year-old diseased beeches (*F. sylvatica*) showing similar symptoms mainly at the trunk bases sometimes reaching the level up to 7 m above the soil. Both pathogens also cause damage to beech seedlings and their mortality. From affected oaks (*Q. robur*) having numerous bark cracks at the base of the bole, in combination with seepage of a brown fluid, and dark stains (which can be visible in transverse sections of the annual rings of stems and branches) *P. cinnamomi* was isolated. The studies curried out in 2005 showed the occurrence of *Phytophthora* species in rivers and streams as well as in standing water. It indicated water as important source of infection with that group of pathogens and showed possibilities of their transferring from one to another country.

Canadian nursery certification program

Barbara Peterson

Horticulture Specialist, Canadian Food Inspection Agency, 59 Camelot Drive Ottawa, Ontario,
K1A 0Y9, Canada

International trade in nursery stock is considered a high risk pathway for the spread of plant pests. Traditionally, phytosanitary certification of nursery stock has been based upon visual inspection of plants prior to shipping. Although visual inspection remains the primary tool of regulatory agencies, it does have limitations as evidenced by several recent introductions of quarantine plant pests, believed to have been associated with propagative plant material certified in the country of export. Examples of pests believed to be imported to North America though propagative material include Plum pox virus, Glassy winged sharpshooter (*Homalodisca coagulata*), and Mulberry long-horned beetle (*Anoplophora chinensis*).

The Canadian Nursery Certification Program (CNCP) is a phytosanitary certification program for Canadian nurseries and greenhouses that export nursery stock to the United States, or within Canada. The CNCP offers an alternative to traditional phytosanitary certification, which is based on final product inspection immediately prior to shipping. The CNCP uses a systems approach to mitigate pest risk. It has several components, including documenting the production and pest management practices, auditing and reviewing the system, and determining pest prevalence during production. Under the CNCP certified facilities implement procedures to ensure that the plants they ship meet the requirements of the CNCP and are free of pests of concern. The Canadian Food Inspection Agency (CFIA) establishes the requirements of the CNCP and conducts audits to verify that plant material certified under the program meets the phytosanitary standard of the importing country and is free of quarantine and regulated non-quarantine pests. In fact, if populations of non-regulated plant pests are not identified and controlled at a certified facility, the facility may be suspended from the CNCP due to non-conformance with the standard. Consequently, nurseries certified under the CNCP will produce plant material that consistently meets the phytosanitary requirements of the importing country.

The International Organization for Standardization (ISO) 9001/4 is based on the principle that applying a management system to an entire production process will result in products that consistently meet, or exceed, the parameters established by the organization. In contrast, products produced under conditions where only the end products are systematically reviewed, are less likely to consistently meet the established parameters. The CNCP uses a systems approach and is based on the IPPC Standard, "*The Use of Integrated Measures in a Systems Approach for Pest Risk Management*" (ISPM 14) and also meets the guidelines of the NAPPO standard, "*Integrated Pest Risk Management Measures for the Importation of Plants for Planting into NAPPO Member Countries*" (RSPM 24).

Physiological effects of *Phytophthora alni* on *Alnus glutinosa*

Hardy Pfanz, Matthias Jüptner, Katja Ries, Christiane Wittmann

Institut für Angewandte Botanik, Universität Duisburg-Essen, Campus Essen,
45117 Essen, Germany

The new hybrid *Phytophthora alni sp. nov*. (Braisier et al. 2004) is spreading in Germany. Along rivers and creeks, surrounding bogs and lakes *Alnus glutinosa* is showing the classic symptoms of leaf drying and crown defoliation as well as stem cankers and bleeding. Many of the infected trees die within the following years (for oak see Jung et al. 2002).

To study the ecophysiological effects of *P. alni* on *Alnus* experiments with intact twigs, intact detached leaves and seedlings were performed in the field and in the laboratory. It was shown that infected trees have less and smaller leaves with a clearly reduced chlorophyll content. Photosynthetic capacity, measured either as electron transport capacity (ETR) via Chl-fluorescence or by CO_2 gas exchange is clearly reduced in infected trees. Also corticular photosynthesis (Pfanz et al. is affected around artificial inoculations of stems. Following anatomical changes around the inoculation, also the chlorophyll content of the inner bark is drastically reduced. ETR measurements underlined this fact. Several centimetres above and below the inoculation spot normal ETR rates around 30-35 µmoles e^{-1} m^{-2} s^{-1} were found within the green inner bark. Closer to the infection rates were reduced via 15 µmoles e^{-1} m^{-2} s^{-1} (1-2 cm) to zero (center of infection). Absorptivity measurements showed clear changes in light absorption of infected and uninfected tissues of the stems. When seedlings or detached twigs were fed with extracts from infected trees, leaf wilting was observed (see also Fromm et al. 1999). Irreversible wilting occurred within 6-24 h. Similar effects were obtained when the seedlings/twigs were fed with sucrose solutions.

An examination of the inspection and enforcement issues related to bark-free specifications for wooden pallets and containers

Charles D. Ray

The Pennsylvania State University, University Park, PA, USA

Of particular concern to the world's wood packaging industry is the lack of a clear definition of the bark requirement actually targeted by the EC 2004/102/EC and related directives. Also, various industries and governmental agencies around the world have interest in determining the impact these and similar directives might have on the wood packaging industry, its customers, and consumers world-wide.

Of interest to operational researchers is the potential efficacy and probability of successful implementation of this type of specification. Related research on process and product specification in international trade has been discussed in the agricultural and food sanitary fields for some time now. Directive 2004/102/EC has interesting characteristics of potential Type 1 and Type 2 inspection error that should be fully explored before final dispensation of the rule is made.

This presentation will provide a qualitative discussion of the relevant inspection issues, empirical data gathered from a recent North American pallet study, present the findings of an exploratory study to determine the Type 1 and Type 2 inspection error rates for bark-free specification of wooden packaging materials, and the resulting impact on the efficacy of the intended phytosanitary measure.

Role of genetic diversity in the introduced *Cryphonectria* – hypovirus pathosystem

Daniel Rigling

WSL Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland,

Chestnut blight caused by the introduced fungus *Cryphonectria parasitica* is widespread in most of the chestnut growing regions of Europe and is still causing significant damage in orchards and forests. The Cryphonectria hypovirus 1 (CHV-1) is responsible for the recovery from the disease in many chestnut populations in Europe (a phenomenon known as hypovirulence), either naturally or after biological control treatments. Chestnut stands where hypovirulence is widespread are characterized by high disease incidence but low disease severity leaving most trees with one or more non-lethal superficial cankers.

Virus transmission among fungal strains is limited by a vegetative incompatibility (vic) system in *C. parasitica* involving at least 6 *vic* loci. In most local subpopulations in Europe, the number of polymorphic *vic* loci and consequently diversity of vegetative compatibility (vc) types is low, which favours spread and persistence of the hypovirus. However, there is a considerable differentiation in respect to polymorphic *vic* loci among European subpopulations, indicating the potential of locally increasing vc type diversity through the introduction of new *vic* alleles from other regions. There is also significant genetic diversity within the hypovirus population in Europe. We have identified several subtypes of CHV-1 with the subtype I being most widespread. The different subtypes also vary in virulence to *C. parasitica* and thus in their ecological fitness and potential as biological control agent.

The observed geographic patterns of genetic diversity support the hypothesis of multiple introductions of both *C. parasitica* and CHV-1 subtypes into Europe. Although sequence evolution within CHV-1 subtype appears to be relatively slow, recombination between CHV-1 subtypes may eventually take place and generate new CHV-1 variants. For *C. parasitica*, there is the risk of an increasing vc type diversity in the post-epidemic populations, which could jeopardize biological control efforts.

Introduced pests and diseases in Switzerland

Roland Engesser, Beat Forster, Franz Meier, Beat Wermelinger, Daniel Rigling

WSL Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland

The monitoring of pests and diseases of forest and ornamental trees in Switzerland is conducted by the Swiss forest protection unit at the WSL. The source of information consists of inquiries, diagnostic samples sent by the forest service and others, and specific surveys and site visits.

The most significant invasive pests on forest or ornamental trees include *Dreyfusia nordmannianae* (balsam woolly aphid on fir, year of introduction ca. 1880), *Gilletteella cooleyi* (on Douglas fir, ca. 1935), *Pulvinaria regalis* (horse chestnut scale, ca. 1992), *Pulvinaria hydrangeae* (‘Hydrangea scale’, ca. 1980), *Corythucha ciliata* (sycamore lace bug, ca. 1975), *Corythucha arcuata* (oak lace bug, ca. 2002), *Xylosandrus germanus* (black stem borer, ca. 1984), Gnathotrichus materiarius (American utilizable wood bark beetle, ca. 1984), *Phyllonorycter robiniella* and *Parectopa robiniella* (two leaf miners on black locust, ca. 1983), *Cameraria ohridella* (horse chestnut leaf miner, ca. 1998), *Hyphantria cunea* (fall webworm, ca. 1991), and *Metcalfa pruinosa* (a flatid panthopper, ca. 1993).

Introduced diseases, which have caused considerable damage in Swiss forests were chestnut blight (caused by *Cryphonectria parasitica,* ca. 1950) and Dutch elm disease (*Ophiostoma ulmi,* ca. 1930; *Ophiostoma novo-ulmi,* ca. 1980). Other introduced pathogens affecting forest or ornamental trees include *Phytophthora cinnamomi* (ink disease of chestnut, ca. 1920) and probably other *Phytophthora* species, *Ceratocystis fimbriata sp. platani* (canker stain of plane, ca. 1985), *Mycosphaerella pini (red band needle blight on pine,* ca. 1989), *Mycosphaerella dearnessii* (brown-spot needle blight of pines, ca. 1995) and *Erwinia amylovora* (fire blight on *Crataegus* sp. and *Sorbus* sp. in forests). *Phytophthora ramorum* was identified for the first time in 2003. So far, *P. ramorum* has only been found on *Viburnum* sp. and *Rhododendron* sp. in parks or nurseries, mostly on imported plants.

Invasive pathogens and pests: an African perspective

Jolanda Roux, Michael J. Wingfield

DST/NRF Centre of Excellence in Tree Health Biotechnology, Tree Protection Co-operative
Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI),
University of Pretoria, Pretoria, South Africa

Trees are crucially important to the people of Africa who use them for many purposes such as for construction, fuel and food production. The utilisation of trees has, however, placed huge pressure on native resources in many parts of Africa. This has necessitated the importation and planting of fast growing, non-native tree species. In some situations these non-native trees form the basis of multi-million dollar paper and pulp industries, providing employment for substantial numbers of people. The increased movement of people and products between continents and countries, and the importation of products from non-native trees and plants has substantially promoted the world-wide spread of pests and diseases. This movement of tree pathogens and pests presents a great threat to native tree species in the importing countries. It also seriously threatens plantations of non-native species in these countries. This is especially true in areas where large monocultures, derived from narrow genetic stock, have been established. Similarly, native pathogens and pests in Africa are adapting to attack non-native plants and these now present a threat to the non-natives in their countries of origin.

Knowledge pertaining to tree pests and diseases is generally lacking in Africa. Where forest entomology and pathology programmes have been established, they have often been interrupted or terminated due to political instability or lack of funding. During the course of the last ten years, with a resurgence of interest in plantation forestry in especially East Africa, many new studies have been initiated to deal with tree pests and diseases on the continent. This has resulted in the identification of numerous previously unreported diseases and pests, as well as in the description of new fungal species from native and non-native trees in Africa. For example, various *Ceratocystis* spp., not known from other continents have been described from Africa during the course of the last decade. Thus, *Ceratocystis albifundus* has been described after it was identified as the cause of a serious wilt and canker disease of non-native plantation grown *Acacia mearnsii* trees in South Africa. More recent studies strongly suggest that *C. albifundus* is native to Africa as it has a high genetic diversity and a broad host range on native African tree species. This pathogen could result in large-scale mortality of *A. mearnsii* and other native Australian plant species, including *Proteaceae*, if it were introduced into Australia. *Sirex noctilio*, a wasp of Eurasian origin, is different in representing a pest that has recently been introduced into South Africa and is now considered the most serious constraint to pine propagation in Southern Africa. This exotic pest and its fungal symbiont *Amylostereum areolatum,* is moving northwards from its original point of introduction and it is only a matter of time before it spreads throughout South Africa and to this country's neighbours.

There are many challenges that face efforts to prevent pests and pathogens from spreading between continents and countries. Once an incursion has occurred, there is little hope of stopping it from spreading locally. This is especially true in Africa where local quarantine procedures are enormously difficult due to funding constraints and relatively open borders between many countries on the continent. We thus recommend, as a first line of defence, that Africa as a continent protects itself against incursions from other continents. Together with this, specific regions on the continent need to be identified to act together forming a secondary barrier to the spread of exotic pests and pathogens. These measures and efforts of individual countries to protect themselves from the incursion of forest pests and diseases should greatly improve the currently threatening situation. While such efforts are being promoted actively, they are commonly hampered by a lack of trained staff, infrastructure, funding and a lack of knowledge regarding the pests and pathogens already on the continent. This emphasises the need to enhance collaboration and sharing of knowledge between African countries and those in other parts of the world to prevent large scale losses of forestry resources on the continent.

Pest risk analysis – a New Zealand perspective

Shiroma Sathyapala, Mike Ormsby, Melanie Newfield

Risk Analysis Group, Pre-Clearance Directorate, Biosecurity New Zealand,
Ministry of Agriculture and Forestry, PO Box 2526, Wellington, New Zealand

Biosecurity for New Zealand has been defined as the exclusion, eradication or effective management of risks posed by pests and diseases to the economy, environment and human health. Risk analysis undertaken for biosecurity is concerned with supporting the risk management decision making process to effectively manage the biosecurity risks to New Zealand associated with the movement of goods or conveyances into New Zealand and the eradication or management of organisms or diseases established in New Zealand. Within New Zealand risk analysis frame work, the scope of pest risk

analysis includes all or selected pathways for the entry of a particular pest or disease or group or class of pests or diseases.
The challenges in pest risk analysis are discussed using case studies including an analysis currently underway on the risks of entry and establishment of selected “high impact” Lepidoptera, and the completed analyses on pinewood nematode and invasive ants on imported sawn timber.

Interactions between woodwasps, their symbiotic fungi and biological control agents have important implications for *sirex* control

Bernard Slippers[1], Brett Hurley[1], Rimvydas Vasiliauskas[2],
Jan Stenlid[2], Michael J. Wingfield[1]

[1] Tree Protection Co-operative Programme, Forestry and Agricultural Biotechnology Institute,
University of Pretoria, Pretoria, South Africa
[2] Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences,
Uppsala, Sweden

The woodwasp *Sirex noctilio* and its *Amylostereum areolatum* fungal symbiont provides an apt example of a complex interaction of biological agents, which have been introduced into the Southern Hemisphere and have resulted in significant damage. We used molecular and phenotypic markers to analyse populations of different woodwasps and their fungi to better understand the interaction between these organisms, the diversity and structure of native populations, and the potential origin of introductions. In some areas, populations of *A. areolatum* are dominated by extensive dispersive clones that arise from wasp dispersal and associated asexual reproductions. This is especially true in Southern Hemisphere pine plantations. In contrast, populations of both *A. chailletii* and *A. areolatum* in some native areas clearly arise from sexually produced basidiospores. The data have shown that a specific genotype of the fungus can be carried by different wasp species, can occur on different tree genera and can fruit in nature. Wasps thus appear to acquire fungal symbionts both vertically and horizontally. The data, furthermore, suggest that there has been more than one introduction of *S. noctilio* into different Southern Hemisphere countries, but there has clearly also been some movement of wasps between these countries. These findings are important, because interactions between biocontrol agents and genotypes of the wasp or fungus have been shown to be highly specific. The distinct populations of wasps and fungi in different geographic areas thus need to be more carefully considered in local *S. noctilio* control programmes. New introductions might also negatively affect successfully implemented biocontrol efforts. Our current work builds on data from the present study and aims to characterise the interaction between specific biocontrol agents and hosts at a local population level.

International regulatory and non-regulatory frameworks on invasive alien species

Wojciech Solarz

Institute of Nature Conservation, Polish Academy of Sciences, 33 Mickiewicza Str.,
PL-31-120 Kraków, Poland

As a result of their deleterious effects upon economy and nature, Invasive Alien Species (IAS) are receiving significant attention from a variety of stakeholders. The presentation will give an overview of recent international developments aiming at reducing the IAS problem. Particular attention will be drawn to initiatives in the field of nature conservation. Among regulatory frameworks, the IAS-related input from the Convention on Biological Diversity (CBD), Bern Convention, Ballast Water Convention and International Plant Protection Convention (IPPC) will be presented. Non-regulatory

frameworks on IAS covered in the presentation will include international organizations: ISSG (Invasive Species Specialist Group), GISP (Global Invasive Species Programme), and GISIN (Global Invasive Species Information Network), as well as projects: ALARM (Assessing LArge scale risk for biodiversity with tested Methods), DAISIE (Delivering Alien Invasive Species Inventories for Europe), and NOBANIS (NOrth-European and BAltic Network on Invasive Species). Gaps and inconsistencies in the international frameworks on IAS will also be briefly discussed.

Nobanis: North-European and baltic network on invasive species

Wojciech Solarz[1], Inger Ravnholt Weidema[2]

[1]Institute of Nature Conservation, Polish Academy of Sciences,
33 Mickiewicza Str., 31-120 Kraków, Poland
[2] Danish Forest and Nature Agency, Haraldsgade 53, DK-2100 Copenhagen,
Denmark irw@sns.dk

The Nordic/Baltic Network on Invasive Alien Species (NOBANIS) is being financed by the Nordic Council of Ministers and the government of Germany. The project has created a gateway to information on invasive alien species by establishing an internet portal – www.nobanis.org. One of the main features on the portal is a dynamic, searchable database which covers the participating countries: Denmark, Estonia, Finland, Faroe Islands, Germany, Greenland, Iceland, Latvia, Lithuania, Norway, Poland, the European part of the Russian Federation and Sweden. More than 10,000 records provide insights on alien species from all environments of the region. Summary data from the database will be used to discuss the immigration, dispersal, establishment and impact of alien species. For the 60 most invasive species the portal presents the scientific community and environmental managers with species profiles/fact sheets written by expert of the region.

NOBANIS provides administrative tools for making the precautionary approach operational in preventing the unintentional dispersal of invasive alien species and mitigating adverse effects of IAS on biological diversity.

NOBANIS established a network for cooperation between competent authorities of the region and contributes to implementing recommendations from the 6th Conference of Parties of the Convention on Biodiversity's and Recommendation No.99/2003 of the Bern Convention to establish regional cooperation to aid in eradication, control and mitigation of ecological effects of invasive alien species.

Alien species in Poland – an online database (www.iop.krakow.pl/ias)

Wojciech Solarz, Damian Chmura, Wiesław Król, Grzegorz Cierlik

Institute of Nature Conservation, Polish Academy of Sciences, 33 Mickiewicza Str. 31-120
Kraków, Poland
solarz@iop.krakow.pl, chmura@iop.krakow.pl, krol@iop.krakow.pl, cierlik@iop.krakow.pl

Collection and dissemination of information on Invasive Alien Species (IAs) are key elements to solve the problems caused by invasions. In 1999, compilation of a database on alien species in Poland started at the Institute of Nature Conservation, Polish Academy of Sciences in Cracow. The database was prepared for the Ministry of Environment. In 2003, part of the data was made accessible via the Internet at www.iop.krakow.pl/ias.

Currently there are 613 alien species of fungi, plants and animals in the database. For most species, information on taxonomical position, synonyms, biology, natural distribution and distribution in Poland is compiled, as well as data on the population numbers and trend, and impact upon native species. Details on the introduction are also gathered, including time, place, number of introduced

individuals and pathway of introduction. The need of the species control is assessed and methods such control are given.
The database is linked to the NOrth-European and BAltic Network on Invasive Species (NOBANIS), Global Invasive Species Information Network (GISIN) and Delivering Alien Invasive Species Inventories for Europe (DAISIE).

Application of synthetic pheromone for catch of males horse chestnut leafminer *Cameraria ochridella* Deschka et Dimić

Sławomir Ślusarski[1], Andrzej Kolk[1], Tomasz Jabłoński[1], Lidia Sukovata[1], Jacek Grodner[2]

[1] Forest Research Institute, Forest Protection Department, Sękocin Las, 05-090 Raszyn, Poland
e-mail: S.Slusarski@ibles.waw.pl
[2] Institute of Industrial Organic Chemistry, Annopol 6, 03-236 Warsaw

Cameraria ohridella Deschka & Dimić (Lepidoptera, Gracillariidae) is the most dangerous pest of the horse chestnut (*Aesculus hippocastanum*, *Hippocastanaceae*) in Europe. In Poland this pest has occurred since 1996 and it has invaded most of horse chestnuts in the whole country. Currently there are few control and monitoring methods in use. In the paper there are presented results of three years investigations on possibilities of use of synthetic pheromones for catching of horse chestnut leaf-miner males. Few types of pheromone traps were tested in this research. Additionally the glue foil trap was tested.

Pine pitch canker – the south African situation

Jane Wright[1], R.J. Ganley[2], E.T. Steenkamp[1], E. Iturritxa[3], R. Ahumada[4],
Brenda D. Wingfield[5], W.F.O. Maras as[6], Michael J. Wingfield[1]

[1] Department of Microbiology and Plant Pathology, Centre of Excellence in Tree Health Biotechnology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa, jane.wright@fabi.up.ac.za
[2] NZ Forest Research Institute Ltd., Private Bag 3020, Rotorua, New Zealand
[3] Neiker, Granja Modelo de Arkaute, Apartado 46, 01080 Vitoria-Gasteiz, Alava, Spain
[4] Bioforest S.A., Camino a Coronel KM. 15S/N, PO Box 70, Concepcion, Chile
[5] Department of Genetics, Centre of Excellence for Tree Health Biotechnology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa
[6] Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council, PO Box 19070, Tygerberg, 7505, South Africa

Pitch canker, caused by the ascomycete *Gibberella circinata* (anamorph *Fusarium circinatum*), is a serious disease of *Pinus spp*. and represents a significant threat to native forests and commercial pine-based forestry. In mature trees the disease results in pitch-soaked wood, crown dieback and stunted growth. In nurseries, it can cause root rot, die-back and dramatic mortality of planting stock, which threatens plantation establishment. In South Africa, all pines grown are non-native and pitch canker has been detected only in nurseries. Evidence based on the epidemiology and genetics of the pathogen has shown that it was probably introduced into South Africa on infected seed, which led to the initial outbreak of the disease. It was subsequently spread to nurseries around the country, most likely on asymptomatic infected seedlings. Currently *F. circinatum* is regarded as the most important pathogen of pine nursery seedlings and it is one of the most serious problems that forestry companies have to contend with. The aim of this study was to gain a better understanding of the global evolution of the pitch canker fungus with particular emphasis on its distribution and spread within South Africa. For this purpose we studied the population biology and phylogeny of the pathogen using multi-locus DNA

sequence information for representative isolates from pitch canker affected areas (South Africa, Spain, Chile, Mexico, Florida, and California). Analyses of the sequences revealed several nucleotide polymorphisms between the five populations, with fewer differences within populations. These data also allowed us to identify sequence signatures that differentiate among the various populations. Our preliminary data suggest that the South African population is most closely related to the populations from California and Florida, which is also reflected by a number of shared unique alleles. This suggests that the disease was introduced into South Africa from the South Eastern United States and not from Mexico as previously hypothesized. However, corroboration of this result will require analysis of more extensive populations from the likely centre of origin for the pitch canker pathogen (i.e. Mexico and Central America). Together with the sequence information for more polymorphic loci, these results will ultimately provide valuable insight into the spread of the pitch canker fungus not only within South Africa but also across the globe.