

A collaborative project to better understand Siricid-Fungal symbioses

Bernard Slippers^{1, 2}, Rimvis Vasiliauskas², Brett Hurley¹, Jan Stenlid² and Michael J Wingfield¹

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 Biotechnology Institute, Uppsala, Sweden

bernard.slippers@fabi.up.ac.za

Abstract

The Forestry and Agricultural Biotechnology Institute, University of Pretoria and the Department of Forest Mycology and Pathology, Swedish University of Agricultural Biotechnology Institute, Uppsala, Sweden are collaborating on a study of the Siricid-Fungal symbiosis, and its parasites. This project aims to address questions in two general areas, namely (a) the evolution and biology of mutualistic symbiosis and (b) the monitoring and control of wood inhabiting pests and pathogens that threaten biodiversity and forest production in introduced and native environments.

Project background

The symbiosis between woodwasps and fungi (Fig. 1)

A mutualistic symbiosis exists between Siricid woodwasps and *Amylostereum* fungi (Talbot 1977, Martin 1992). The

relationship between these organisms is specialised and obligatory species specific, at least for the insects. The principle advantage for the fungus is that it is spread and inoculated into suitable wood substrates during wasp oviposition. In turn, the fungus rots and dries the wood, providing a suitable environment, nutrients and enzymes to the developing insect larvae.

The burrowing activity of the Siricid larvae and fungal white rot of the wood make this insect-fungus symbiosis potentially harmful to its conifer host trees. However, in the northern hemisphere, where the Siricidae are native, the insect is of little economic importance, except during times of increased stress due to other factors (Spradbery & Kirk 1978). Here a natural balance exists between the insect-fungus complex, its natural parasites and host trees as long as the trees are generally healthy. These organisms have been studied widely in Europe to understand their fascinating biology.

Amylostereum spp. are Basidiomycetes that are heterothallic and have a tetrapolar nuclear state (Boidin & Lanquetin 1984). Such a mating system increases outcrossing

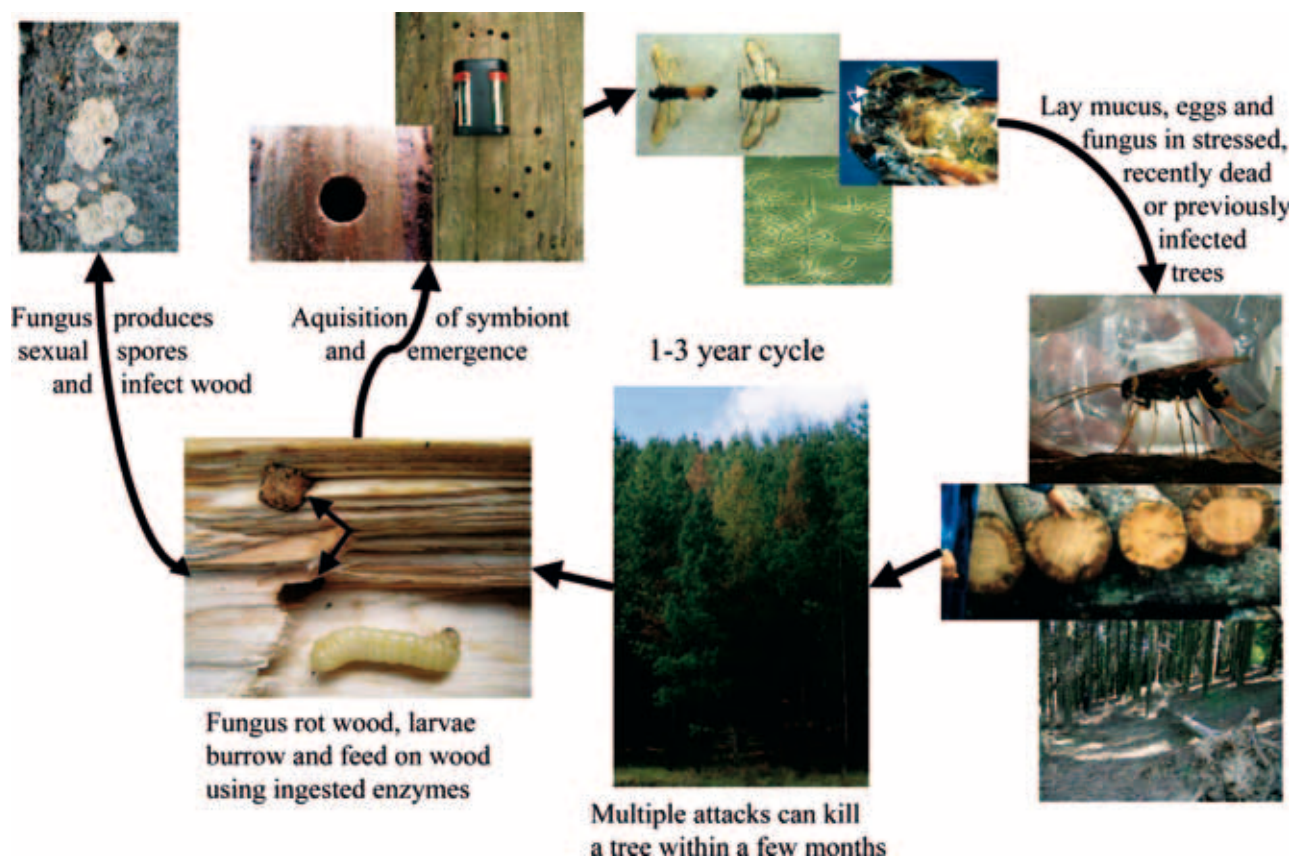


Fig. 1. Life-cycle of Siricid woodwasps and their *Amylostereum* symbiotic fungi.

and thus normally also population diversity. The *Amylostereum* spp. are, however, also spread by woodwasps in the form of asexually produced oidia (thus genetically identical) Vasiliauskas et al. 1998).

In the northern hemisphere clonal lines of *A. areolatum* and *A. chaillietii* are preserved over time and occur over large areas as a result of the spread of oidia of by woodwasps (Vasiliauskas et al. 1998, Thomsen & Kock 1999, Vasiliauskas & Stenlid 1999). This situation is even more dramatic in the southern hemisphere where a single vegetative compatibility group (VCG) dominates populations of *A. areolatum* associated with *S. noctilio* (Slippers et al. 2001). Isolates from South Africa, Brazil and Uruguay represent the same VCG. This VCG in turn was partially compatible with isolates from New Zealand and Tasmania. These results suggest that the spread of *Sirex* through the southern hemisphere during this century has taken place among the continents and countries of this region, rather than by separate introductions from the northern hemisphere. The results, further, indicate that *A. areolatum* in the southern hemisphere spreads exclusively asexually through its association with *S. noctilio*. No sporocarps of *A. areolatum* have thus far been found in the southern hemisphere.

Woodwasp-fungal symbionts as forest pests and their control

There is an increasing number of exotic pest and pathogen invasions that threaten the world's ecosystems (Bright 1998, Wingfield et al. 2001). Many of these introductions have had or are having catastrophic outcomes. The long-term sustainability of native forest and forestry industries will depend on the capacity to effectively deal with such introduced insect pests and pathogens.

Forests in Europe are increasingly at risk from newly introduced pathogens, continued human pressure and alteration of habitat, as well as global weather changes. Evidence of this has been numerous emergences of disease outbreaks or species 'declines' across Europe. Dutch-elm disease and Oak decline in central and southern Europe, *Fraxinus* decline in northern Europe, *Pinus* dieback in various areas in Europe, *Ostrya* decline in southern Europe, etc. The current amount of freshly dead wood (75 mil m³) in Sweden following the storm of January 2005 adds to this risk for native forests as many Siricids prefer such material to breed in (Spradbery & Kirk 1978). Significant increases in Siricid populations, coupled with the pressures mentioned above, can hold significant risks for attacks on stored (unharvested) timber and standing trees weakened by other pests (e.g. bark beetles and *Armillaria* root rot). Such a situation exists in parts of Switzerland (Dr. U. Heiniger, pers. comm.).

Sirex noctilio and *A. areolatum* have been introduced into various southern hemisphere countries and, recently, to the USA (where it is currently viewed as a potential threat to forest health) (Slippers et al. 2003, Hoebeke et al. 2005). In contrast to the native range, these symbiotic organisms have caused extensive mortality in exotic pine

plantations in the southern hemisphere (Chou 1991, Madden 1988). Despite the costly efforts to monitor and control the wasp and fungus during the previous century, the pest complex continues to kill significant numbers of trees and spread to previously unaffected areas in Australia, South Africa and South America. In many of these regions this pest complex is considered to be the biggest threat to pine forestry operations.

Sirex noctilio is most effectively controlled through biological control agents such as the nematode *Deladenus siricidicola* and some parasitic wasp species, in combination with silvicultural practices aimed at reducing tree stress (Neumann et al. 1987, Haugen 1990). The nematode is, however, the main form of control. *Deladenus siricidicola* has a closely co-evolved and integrated life cycle with both the wasp and fungal symbiont (Fig. 2). For this reason, the efficiency of biocontrol programmes is often affected by the specific nematode strain or fungal strain involved. Wasp parasites are currently underused in many countries due to incomplete information from native ranges and weak application strategies.

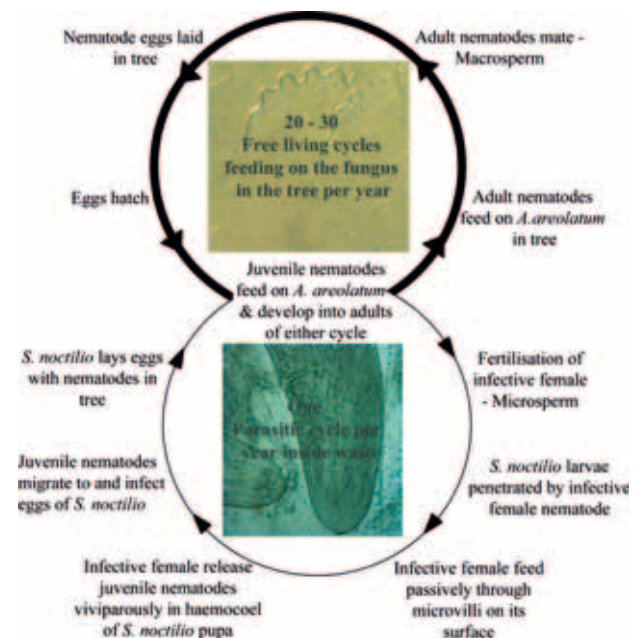


Fig. 2. Bicyclic life cycle of the *Sirex* biocontrol nematode, *Deladenus siricidicola*. (Adapted from Bedding 1972, *Nematologica*)

General questions addressed in the project

Molecular techniques have only recently been applied to questions pertaining to *Amylostereum* taxonomy, phylogeny and population structures (Vasiliauskas et al. 1999, Slippers et al. 2000, 2002, Tabata et al. 2000). These studies have clarified previous hypotheses that were based on morphological and mating studies, regarding the relationships among *Amylostereum* spp. They have also raised new and challenging questions regarding the identity of the fungal isolates associated with certain woodwasps. From these preliminary observations there appear to be cryptic

speciation that have been overlooked using traditional methods of identification. On a higher taxonomic level, the relationship of *Amylostereum* to other Basidiomycetes is currently unsure due to contradictory literature reports (Slippers et al. 2003).

A study of the population structure of *Amylostereum* fungi from many parts of the world, using both VCG's and molecular markers, will give valuable insight into the geographical origin and spread of these fungi, as well as their associated Siricid wasps. Such data have already identified patterns of spread amongst countries in the southern hemisphere and between some local populations in Scandinavia (Vasiliauskas et al. 1998, Thomsen & Koch 1999, Vasiliauskas & Stenlid 1999, Slippers et al. 2002). Phylogeographic data is, however, lacking for most of natural distribution of Siricids and their fungi. The northern hemisphere origins of southern hemisphere populations of *Sirex* and *Amylostereum* are not known, despite its importance for selection of control agents.

Despite detailed studies of the symbioses between Siricid woodwasps and their fungal symbionts, many fundamental questions remain unanswered. For example, it is thought that vertical transmission (from mother to daughter) predominates. However, the numerous wasp species apparently carrying the same fungal species indicate some level of horizontal transfer of the symbiont between wasp species. The importance of such data is illustrated by the lack of any explanation of the fundamental differences in population structures of *A. areolatum* (highly clonal) and *A. chaileitii* (almost indistinguishable from population structures of other basidiomycetes spreading through sexual spores). Furthermore, there is no co-evolutionary or phylogeographic data on which to infer the evolutionary development of the symbiosis. The lack of this information also excludes the comparison of this symbiosis with other symbiotic systems.

Siricid-like wasps are known from the Jurassic period (more than 150 mya) Rasnitsyn 1988). Parallels between the Siricid-fungal symbiosis and other independently derived symbioses are likely to reveal evolutionary factors that are important for the development and stability of such partnerships. Such a co-evolved system also presents important opportunities to study comparative rates of molecular evolution in different symbiotic partners, and non-symbiotic relatives, as well as addressing general questions of the adaptive significance of sex (Herre et al. 1999).

The artificial selection during mass rearing of biological control agents in control programmes can lead to severe bottlenecks in populations of these organisms. This will severely reduce population diversity in the control organisms, which will reduce their ability to respond to changes in the environment or host. During the nematode rearing process the accidental selection of less infective strains of *D. siricidicola* has led to a temporary breakdown of the biological control programme in Australia, resulting in huge damages (Haugen 1990). Despite these dangers, there is currently no data or methods available to study popula-

tions, compare strains or track changes in populations of the biological control organisms.

In order to conduct this study, collections of populations of wasps, fungi and biocontrol agents are needed to represent the native occurrence of these organisms, as well as areas where they have been introduced. Collected samples from the southern hemisphere (Argentina, Brazil, Australia, South Africa) and Europe (Austria, Denmark, Great Britain, Italy, Greece, Norway, Sweden, Switzerland) have been made in collaboration with various other researchers and research organization. This material is supplemented from international culture collections and herbaria (Canada, France, Germany, Japan, Russia, USA). As part of collecting efforts, potential attractants and methods have been identified to catch woodwasps. These collections are ongoing.

Conclusion

It is hoped that the project will help unravel the evolutionary causes and consequences of woodwasp-fungal symbiosis. Such basic information will contribute to understanding fungal-insect symbiosis, as well as symbiosis as a general biological theme influencing evolution of organisms. In addition, such data will provide practical assistance to monitoring and controlling programs of introduced population of Siricid woodwasps and their symbiotic fungi. It will also help to characterize patterns of natural and human-mediated spread of these insects. From these data, the project should also contribute to the growing body of knowledge concerning international movement and control of pests and pathogens, to help prevent recurrence of such events.

Acknowledgements

We wish to thank the Tree Protection Co-operative Programme, Forestry SA, University of Pretoria, Swedish University of Agricultural Sciences, the SIDA-NRF South African – Swedish Research Partnership Programme, NRF Postdoctoral Programme and the Skye Foundation for financial support for this project.

References

- Boidin J & Lanquetin P 1984. Le genre *Amylostereum* (Basidiomycetes) intercompatibilités partielles entre espèces allopatriques. *Bull Soc Mycol France* 100: 211–236.
- Bright C 1998. Life out of bounds. Bioinvasion in a borderless world. New York: WW Norton.
- Chou KKS 1991. Perspectives of disease threat in large-scale *Pinus radiata* monoculture – the New Zealand experience. *Eur J For Path* 21: 71–81.
- Haugen DA 1990. Control procedures for *Sirex noctilio* in the Green Triangle: Review from detection to severe outbreak (1977–1987). *Aust For* 53: 24–32.
- Herre EA, Knowlton N, Mueller UG & Rehner SA 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends Ecol Evol* 14: 49–53.
- Hoebeke ER, Haugen DA & Haack RA 2005. *Sirex noctilio*: discovery of a Palearctic siricid woodwasp in New York. *Newsl Michn Entomol Soc* 50: 24–25.
- Madden JL 1988. *Sirex* in Australasia. In: *Dynamics of Forest Insect Populations. Patterns, Causes, Implications*. Berryman AA (ed), Plenum Press, New York, pp. 407–429.
- Martin MM 1992. The evolution of Insect-Fungus associations: From contact to stable symbiosis. *Am Zool* 32: 593–605.
- Neumann FG, Morey JL & McKimm RJ 1987. The *Sirex* woodwasp in Victoria. *Depart Conserv, For Lands, Victoria. Bull* 29, 41pp.
- Rasnitsyn AP 1988. An outline of evolution of the hymenopterous insects (order Vespida). *Oriental Insects* 22: 115–145.
- Slippers B, Wingfield MJ, Wingfield BD & Coutinho TA 2000. Relationships among *Amylostereum* species associated with Siricid woodwasps inferred from mitochondrial ribosomal DNA sequences. *Mycologia* 92: 955–963.
- Slippers B, Wingfield MJ, Wingfield BD & Coutinho TA 2001. Population structure and possible origin of *Amylostereum areolatum* in South Africa. *Plant Pathol* 50: 206–210.
- Slippers B, Wingfield BD, Coutinho TA & Wingfield MJ 2002. DNA sequence and RFLP data reflect relationships between *Amylostereum* species and their associated wood wasp vectors. *Mol Ecol* 11: 1845–1854.
- Slippers B, Coutinho TA, Wingfield BD & Wingfield MJ 2003. The genus *Amylostereum* and its association with woodwasps: a contemporary review. *S Afr J Sci* 99: 70–74.
- Spradbery JP & Kirk AA 1978. Aspects of the ecology of siricid woodwasps (Hymenoptera: Siricidae) in Europe, North Africa and Turkey with special reference to the biological control of *Sirex noctilio* F. in Australia. *Bull Entomol Res* 68: 341–359.
- Tabata M, Harrington TC, Chen W & Abe Y 2000. Molecular phylogeny of species in the genera *Amylostereum* and *Echinodontium*. *Mycoscience* 41: 585–593.
- Talbot PHB 1977. The *Sirex*-*Amylostereum*-*Pinus* association. *Ann Rev Phytopathol* 15: 41–54.
- Thomsen IM & Koch J 1999. Somatic compatibility in *Amylostereum areolatum* and *A. chailletii* as a consequence of symbiosis with siricid woodwasps. *Mycol Res* 103: 817–823.
- Vasiliauskas R & Stenlid J 1999. Vegetative compatibility groups of *Amylostereum areolatum* and *A. chailletii* from Sweden and Lithuania. *Mycol Res* 103: 824–829.
- Vasiliauskas R, Johannesson H & Stenlid J 1999. Molecular relationships within the genus *Amylostereum* as determined by internal transcribed spacer sequences of the ribosomal DNA. *Mycotaxon* 71: 155–161.
- Vasiliauskas R, Stenlid J & Thomsen IM 1998. Clonality and genetic variation in *Amylostereum areolatum* and *A. chailletii* from Northern Europe. *New Phytol* 139: 751–758.
- Wingfield MJ, Slippers B, Roux J & Wingfield BD 2001. Worldwide movement of forest fungi, especially in the Tropics and Southern Hemisphere. *BioScience* 51: 134–140.