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Population structure and possible origin of *Amylostereum areolatum* in South Africa

B. Slippers*†, M. J. Wingfield, T. A. Coutinho and B. D. Wingfield

Forestry and Agricultural Biotechnology Institute (FABI), Faculty of Agricultural and Biological Sciences, Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa 0002

The woodwasp, *Sirex noctilio*, and its symbiotic fungus, *Amylostereum areolatum*, cause extensive damage to pine plantations in the Southern Hemisphere. *S. noctilio* was first reported from South Africa in 1994. In this study, the population diversity of *A. areolatum* isolates from South Africa, South America, Australasia and Europe was determined by vegetative incompatibility testing. All 108 South African and 26 South American isolates belonged to the same vegetative compatibility group (VCG). This VCG showed a weak incompatibility reaction with the single Tasmanian and single New Zealand isolates tested. This VCG differed from VCGs from Europe. It also differed from isolates associated with the biocontrol nematode, *Deladenus siricidicola*, which is produced in Australia. It is concluded that the South African and South American populations of *A. areolatum* share a common origin.

Keywords: *Amylostereum*, population diversity, *Sirex* woodwasp, vegetative incompatibility

Introduction

Sirex noctilio and its symbiotic fungal associate, *Amylostereum areolatum*, are indigenous in the temperate regions of the Northern Hemisphere and are thought to be of Eurasian origin (Benson, 1943; Morgan, 1968; Spradbery & Kirk, 1978). In these regions, this insect–fungus complex is considered a secondary problem of little economic importance (Chrystal, 1928; Hanson, 1939; Hall, 1978; Spradbery & Kirk, 1978). During the 20th century, *S. noctilio* and *A. areolatum* have become established in New Zealand, Australia, South America and South Africa (Madden, 1988; Baxter *et al*., 1995; Reardon *et al*., 1995; Tribe, 1995; Slippers *et al*., 2000), causing great economic losses in *Pinus radiata* plantations. The large monocultured stands of pine, favourable bioclimatic conditions and general absence of natural enemies of *Sirex* have all contributed to elevating this pest complex to primary status (Spradbery & Kirk, 1978; Madden, 1988; Haugen, 1990; Neumann & Marks, 1990; Chou, 1991).

Gaut (1969) used biological species tests and protein gel electrophoresis to show that the fungus associated with *S. noctilio* in Australasia is *A. areolatum*. He also found that isolates with the same geographical origin had homologous protein and enzyme patterns and this could be a useful tool in tracing the origins of the introduced symbiotic woodwasps (Gaut, 1970; Talbot, 1977).

A simple method of recognizing genotypes in fungi is through the use of nonself rejection or vegetative incompatibility (Rayner, 1991; Worrall, 1997). Various authors have recently reported the existence of extensive clonal lineages or vegetative compatibility groups (VCGs) in *A. areolatum* (Vasiliauskas *et al*., 1998; Thomsen & Koch, 1999; Vasiliauskas & Stenlid, 1999). *A. areolatum* is a heterothallic fungus with a tetrapolar nuclear state, and heterokaryotic isolates arising through pairing of primary mycelium from basidiospores would represent separate genetic entities (Boidin & Lanquentin, 1984). The basidiomata are rare in the Northern Hemisphere and have never been reported from the Southern Hemisphere (Thomsen, 1998). These fungi are spread through asexually produced arthrospores that are carried in mycangia of female siricid woodwasps, which result in clonal lines that are widely spread and persist for long periods of time (Vasiliauskas *et al*., 1998; Thomsen & Koch, 1999; Vasiliauskas & Stenlid, 1999). The origins of introduced isolates of *A. areolatum* could be determined by tracing these clonal lines.

Vegetative compatibility was used to investigate the population structure of *A. areolatum* in South Africa and South America. These populations are also compared to other isolates of *A. areolatum*, including those used to rear the biocontrol nematode *Deladenus*

*To whom correspondence should be addressed.

†E-mail: bernard.slippers@fabi.up.ac.za

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siricidicola (Bedding, 1995), to determine the possible spread and origin of *S. noctilio* in the Southern Hemisphere.

Materials and methods

Collection and isolation of fungal isolates

Isolates of the fungal symbiont of *S. noctilio* in South Africa and South America were made from mycangia of female wasps or from wood around tunnels of the wasp larvae, onto selective agar (10 g L^{-1} malt extract, 15 g L^{-1} agar, 2 p.p.m. benomyl powder and 100 p.p.m. streptomycin) (Hsiau, 1996), malt yeast agar (MYA) (20 g L^{-1} malt extract, 2 g L^{-1} yeast extract and 15 g L^{-1} agar) or pine extract MYA (PMYA) (150–200 g pine wood L^{-1} was double autoclaved, strained and this extract used for MYA).

S. noctilio wasps were collected during the flight seasons from 1994 to 1998 from plantations around Cape Town, South Africa, Brazil and Uruguay (Table 1). Isolates of *A. areolatum* were made from cultures of the nematode *D. siricidicola* exported from Australia as a biological control agent to South Africa during 1995 (Table 1). Authenticated isolates of *A. areolatum* from Europe and Australasia were obtained from various culture collections (Table 1). All isolates were maintained on MYA or PMYA at 25°C and were stored on MYA slants at 4°C.

Vegetative compatibility

Pairings of heterokaryons were performed by placing two plugs from actively growing cultures, 1 cm apart, on PMYA. Reactions were scored after 2–3 weeks, but cultures were also incubated for longer periods to confirm that the reaction did not change. Positive controls and negative controls were run in all tests. Reactions were scored as compatible or incompatible. An intermediate reaction was also observed where opposing cultures formed sparse growth in the interaction zone, but not the brown discoloration characteristic of incompatible reactions. Pairings were repeated twice.

Pairings to determine VCGs occurred in different phases. Firstly, South African isolates collected during each season were paired in all possible combinations, for each season; selected isolates from each season were then paired against each other. Secondly, the 25 Brazilian isolates were paired in all possible combinations; five of these

Table 1 Isolates of *Amylostereum areolatum* used in heterokaryon pairings

Culture	Host or source of isolation	Origin	Date isolated	Isolated by
European isolates				
CBS 305·82	Unknown	France	1964	J. Boiden
CBS 334·66	From *Picea abies*	Germany	1967	Dimitri
L204 (A2)[a]	Wood of wounded *P. abies*	Lithuania	1994	R. Vasiliauskas
L236 (DK-A)[a]	Wood of wounded *P. abies*	Lithuania	1995	R. Vasiliauskas
DK37 (DK-A)[a]	Fruiting body on *P. abies*	Denmark	1993	I.M. Thomsen
DK782 (DK-B)[a]	Fruiting body on *P. abies*	Denmark	1987	J. Koch
S225 (A2)[a]	Wood of wounded *P. abies*	Sweden	1994	R. Vasiliauskas
S227 (A2)[a]	Wood of wounded *P. abies*	Sweden	1994	R. Vasiliauskas
Australasian isolates				
Waite Inst. 6195	Mycangium of *Sirex noctilio*	Tasmania, Australia	1962	Unknown
DAOM 21785	*Pinus radiata* infested by *S. noctilio*	New Zealand	Unknown	G.B. Rawlings
8 isolates	From nematode cultures from CSIRO	Australia	1995	B. Slippers
South American isolates				
25 isolates	Mycangia of *S. noctilio*	Brazil	1997	B. Slippers
1 isolate	Mycangia of *S. noctilio*	Uruguay	1998	B. Slippers
South African isolates				
1994/1995				
4 isolates	*P. radiata* wood infested by *S. noctilio*	South Africa	1994	M.J. Wingfield
2 isolates	Mycangia of *S. noctilio*	South Africa	1995	B. Slippers
1995/1996				
56 isolates	Mycangia of *S. noctilio*	South Africa	1996	B. Slippers
1996/1997				
45 isolates	Mycangia of *S. noctilio*	South Africa	1997	B. Slippers
1997/1998				
1 isolate	Mycangium of *S. noctilio*	South Africa	1998	B. Slippers

[a]VCGs as reported by Thomsen & Koch (1999) and Vasiliauskas & Stenlid (1999).

Table 2 Vegetative compatibility between groups of isolates of *Amylostereum areolatum* from various origins[a]

Isolates	Origin[b]	South Africa	South America	New Zealand	Australia	Nematode[c]	Europe
108	South Africa	+					
26	South America	+	+				
1	New Zealand	±	±	+			
1	Australia	±	±	+	+		
8	Nematode[c]	–	–	–	–	+	
8	Europe	–	–	–	–	–	±

[a]Isolates were paired on PMYA reactions and were scored as compatible (+), incompatible (–) or intermediate (±).
[b]Isolates from each group belong to one VCG, except the European isolates that represent different VCGs (Table 1).
[c]Isolated from nematode cultures from CSIRO (Australia), but possibly originate from Europe.

were paired against the Uruguayan isolate; 10 Brazilian and the Uruguayan isolate were paired against the South African isolates representing each of the different seasons. Lastly, seven representative South African isolates and four South American isolates were paired against authenticated isolates from Europe (CBS 305·82, CBS 334·66, L204, L236, DK782, DK37, S225 and S227), New Zealand (DAOM 21785), Tasmania (Waite Inst. 6195) and from nematode cultures (A3, A4, A6–A11). The authenticated isolates were also paired with each other in all possible combinations.

Results

All isolates collected within South Africa during the course of four seasons (1994/1995–97/1998) were somatically compatible. Colony morphologies and interactions sometimes appeared to be less uniform when compared to controls, but the mycelia always intermingled freely. Similarly, all South American isolates grew as a single entity when paired in various combinations. All South African and South American isolates were also vegetatively compatible with each other (Table 2). South African and South American cultures showed minor variations in colony morphologies, but incompatible reactions were never observed.

The isolates from New Zealand and Tasmania, Australia, were fully compatible with each other (Table 2). These isolates showed an intermediate reaction when paired against isolates from South Africa and South America (Table 2). This was in contrast to the incompatible reactions between these isolates (from New Zealand, Tasmania, Australia, South Africa and South America) and any of the other isolates used in the study. The cultures, however, did not grow as an entity as was seen in the positive controls. Colony morphologies of isolates DAOM 21785 and Waite Inst. 6195 were similar to those of isolates from South America and South Africa.

All pairings between South African and South American isolates and isolates from nematode cultures were strongly incompatible (Table 2). Pairings between representative isolates from the single South African/South American VCG and European isolates were strongly incompatible (Table 2). Isolates from the nematode cultures were also incompatible with these isolates from Europe (Table 2). The CBS cultures from Europe were incompatible with each other, as well as with the European isolates DK37 (DK-A) from Denmark and two each from Lithuania and Sweden (Table 1).

Discussion

This study shows that *A. areolatum* isolates from South Africa, collected over four seasons, represent a single VCG, suggesting a limited introduction of *S. noctilio* into the country. The introduction of *Sirex* into South Africa, however, was not necessarily by a single female. Thomsen & Koch (1999) showed that wasps from the same tree usually carry isolates of *Amylostereum* of the same VCG. The introduction could also have taken place more than once, but from the same source. As the *A. areolatum* VCG from South Africa and South America are the same, *S. noctilio* in these two regions either share a common origin, or the woodwasp was introduced into South Africa from South America.

The VCG of *A. areolatum* in South Africa has persisted over the four seasons 1994/1995–1997/1998. In Brazil and Uruguay, where there is also a single VCG, *S. noctilio* has been known since the 1980s (Reardon *et al.*, 1995). These results indicate that vegetative reproduction in its symbiosis with *S. noctilio* is the predominant or only form of reproduction of *A. areolatum* in South Africa and South America. Basidiocarps of *A. areolatum* have not been reported in these countries.

Although incompatible reactions were never observed between isolates from South Africa and South America, interactions were sometimes not as uniform as seen in the positive controls. Variability in the interactions between isolates of *Amylostereum* spp. has also been noted by Thomsen & Koch (1999) and Vasiliauskas *et al.* (1998), as well as between isolates from populations of other basidiomycetes (Adams & Roth, 1967; Coates *et al.*, 1981; Boddy & Rayner, 1982; Rayner & Turton, 1982; Stenlid, 1985). General conclusions from those studies were that the rejection reaction was weaker between more closely related isolates than between unrelated isolates or isolates separated by larger geographical distances.

Isolates of *A. areolatum* from New Zealand and Tasmania were vegetatively compatible with each other and similar to the South African–South American VCG. As *Sirex* was reported in New Zealand around 1900 and in Tasmania in the early 1950s, these results suggest that the introduction of *Sirex* into Tasmania was either from New Zealand or both introductions have a common origin. Isolates from South Africa and Brazil were not fully compatible with the isolates from New Zealand and Tasmania, but were more closely related to them than to any of the other isolates used in the study. The isolate from Tasmania has been in culture since 1962 and such extensive subculturing could also explain the slight differentiation seen here. The possibility that *Sirex* was introduced to South America and South Africa from Australasia is thus not ruled out.

The *D. siricidicola* nematodes used in biocontrol programmes are reared in Australia on cultures of *A. areolatum*, from where they are imported by other countries in the Southern Hemisphere. The origin of these *A. areolatum* isolates is uncertain. They may have been collected from the field in Australasia, or originated from earlier isolations of the nematode in Europe, possibly from *S. juvencus* (R.A. Bedding, CSIRO, Australia, personal communication). The strong antagonism between the isolates from the nematode cultures and isolates from other countries in the Southern Hemisphere has two possible consequences. Firstly, it might affect nematode feeding and reproduction when introduced to new countries or areas. This would be consistent with suggestions that certain isolates of *A. areolatum* from the field in Australia are better than others for rearing the nematode (R.A. Bedding, CSIRO, Australia, personal communication). Secondly, it implies that a different genetic entity of *A. areolatum* has been introduced into South Africa and South America with the nematode, which might influence the population structure of *A. areolatum* in these countries in the future.

In this study, the vegetative compatibility test was useful in suggesting the original source of introduction of *Sirex* to the Southern Hemisphere. One advantage of this technique is that it allows for relatively easy and inexpensive screening of a large number of isolates for genetic similarities and dissimilarities (Stenlid, 1985). Some disadvantages, however, are that whilst mycelial incompatibility clearly constitutes genetic difference, mycelial compatibility does not necessarily constitute clonality or somatic compatibility (Worrall, 1997). Although indications from work of Thomsen & Koch (1999) and Vasiliauskas *et al*. (1998) are that the genetic entities represented by a VCG in *A. areolatum* are clonal, the true clonality of the VCG found in South Africa and South America cannot be implied from the present results.

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