FUNGAL BIOLOGY XXX (2015) 1–10

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/funbio

Multilocus genotyping of *Amylostereum* spp. associated with *Sirex noctilio* and other woodwasps from Europe reveal clonal lineage introduced to the US

Louela A. CASTRILLO[a,*], *Ann E. HAJEK*[a], *Juan A. PAJARES*[b], *Iben M. THOMSEN*[c], *György CSÓKA*[d], *Shawn C. KENALEY*[e], *Ryan M. KEPLER*[a,1], *Paula ZAMORA*[f], *Sergio ANGELI*[g]

[a]*Department of Entomology, Cornell University, Ithaca, NY 14853, USA*
[b]*Sustainable Forest Management Research Institute, University of Valladolid, Palencia 34071, Spain*
[c]*Department of Geosciences and Natural Resource Management, University of Copenhagen, Frederiksberg 1958, Copenhagen, Denmark*
[d]*Department of Forest Protection, Forest Research Institute, Mátrafüred 3232, Hungary*
[e]*Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA*
[f]*Calabazanos Forest Health Center, Castile and Leon, Palencia 34190, Spain*
[g]*Faculty of Science and Technology, Free University of Bozen-Bolzano, Bolzano 39100, Italy*

ARTICLE INFO

Article history:
Received 12 November 2014
Received in revised form
12 February 2015
Accepted 4 March 2015
Corresponding Editor:
Joseph W. Spatafora

Keywords:
Fungal symbiont
Genetic diversity
Invasive pest
Siricid woodwasps
Somatic incompatibility

ABSTRACT

Sirex noctilio is a woodwasp of Eurasian origin that was inadvertently introduced to the southern hemisphere in the 1900s and to North America over a decade ago. Its larvae bore in *Pinus* spp. and can cause significant mortality in pine plantations. *S noctilio* is associated with a symbiotic white rot fungus, *Amylostereum areolatum*, which females inject into trees when they oviposit and which is required for survival of developing larvae. We compared the genetic diversity of *A. areolatum* isolated from *S. noctilio* and other woodwasps collected from Europe and from northeastern North America to determine the origin of introduction(s) into the United States. Multilocus genotyping of nuclear ribosomal regions and protein coding genes revealed two widespread multilocus genotypes (MLGs) among the European samples, one of which is present in the US. The other two MLGs associated with *S. noctilio* in the US represented unique haplotypes. These latter two haplotypes were likely from unrepresented source populations, and together with the introduced widespread haplotype reveal multiple *A. areolatum* MLGs introduced by *S. noctilio* and indicate

* *Corresponding author*. Tel.: +1 607 255 7008; fax: +1 607 255 1132.
E-mail address: lac48@cornell.edu (L. A. Castrillo).

Abbreviations; IGS, intergenic spacer region; ITS, internal transcribed spacer region; mtssu, mitochondrial small subunit; RPB2, DNA directed ribosomal polymerase II subunit; tef1, translation elongation factor alpha 1

[1] Current address: Systematic Mycology and Microbiology, USDA ARS, Beltsville, MD.

http://dx.doi.org/10.1016/j.funbio.2015.03.004

possible multiple *S. noctilio* introductions to North America from Europe. Our results also showed a lack of fidelity between woodwasp hosts and *Amylostereum* species.

Introduction

Amylostereum areolatum (Basidiomycetes: Russulales) is a saprotrophic decay fungus that can be found in stumps or logs of various conifers and is often effectively injected by woodwasps of the genus *Sirex* spp. (Hymenoptera: Siricidae) into living trees. The fungus is carried in a pair of specialized intersegmental mycangia located at the base of the female woodwasp's ovipositor and opening into the oviducts. Fragments of the fungus, or arthrospores, are deposited into host trees with the woodwasp eggs during oviposition. Larval nutrients are derived from wood decomposed by the fungus (Thompson *et al.* 2014). Surveys on the genetic diversity of this fungus collected from woodwasps, basidiocarps or wounded trees from Denmark, Lithuania, and Sweden and tested using somatic or vegetative compatibility grouping (VCG) assays have revealed widespread and conserved clonal populations (Thomsen & Koch 1999; Vasiliauskas & Stenlid 1999). These results along with the general low occurrence of *A. areolatum* fruiting bodies on logs versus its prevalence in wounded trees in some locations within central and northern Europe indicate that this species uses primarily asexual reproduction due to its close association with woodwasp hosts (Thomsen & Koch 1999; Vasiliauskas & Stenlid 1999).

In Europe, *A. areolatum* is associated with the woodwasps *Sirex juvencus* and *Sirex noctilio* (Gaut 1970). Two other species of *Amylostereum* are also associated with siricid woodwasps: *Amylostereum chailletii* is carried by *Sirex cyaneus*, *Sirex areolatus*, and *Urocerus* spp. in Europe (Gaut 1970; Tabata *et al.* 2000), while *Amylostereum laevigatum* is associated with *Urocerus antennatus* and *Urocerus japonicus* in Japan (Tabata & Abe 1997, 1999).

Amylostereum areolatum benefits from its association with the woodwasp *S. noctilio* in multiple ways: the insect transports the fungus to suitable hosts and also injects the host with a venom gland secretion that facilitates lethal infection by the fungus (Bordeaux *et al.* 2014). The venom, noctilisin, induces a variety of physiological changes in the host resulting to weakened defense, enabling the fungus to grow and colonize the surrounding wood (Bordeaux *et al.* 2014). This woodwasp attacks primarily *Pinus* (99 %) but has been found to also infest *Abies* and *Picea* (Spradbery & Kirk 1978).

In the last century, *S. noctilio* was introduced, likely via the movement of infested logs or packing materials, to the southern hemisphere, being found in New Zealand in the 1900s and Australia in 1952, South America in 1980, and South Africa in 1994 (Gilbert & Miller 1952; Morgan & Stewart 1966; Madden 1988; Tribe 1995; Iede *et al.* 2010). This woodwasp is of little economic importance across its native distribution in Eurasia and North Africa as it attacks stressed, dying, or recently dead hosts (Spradbery & Kirk 1978). *S. noctilio*, however, has become a major pest in areas of introduction. Woodwasp outbreaks in exotic *Pinus radiata* plantations in the southern hemisphere in the 1940s and 1950s resulted in up to 80 % tree mortality, making this woodwasp a serious threat to the softwood industry (Madden 1988). *S. noctilio* was first detected in North America in New York in 2004 (Hoebeke *et al.* 2005), and since then has been collected in six other states and also in the provinces of Ontario and Quebec in Canada (NAPIS, 2014), causing serious concerns for the possible impact of this invasive pest on forest ecosystems in North America. In addition, the surveillance for *S. noctilio* in the US forests has decreased in recent years; thus, its present geographic distribution likely is underestimated.

Current biological control strategies in the southern hemisphere utilize the nematode *Deladenus siricidicola*, which parasitizes *S. noctilio* as well as feeds on its fungal symbiont (Bedding 2009). The nematode life cycle has alternating parasitic and mycetophagous phases, and the latter phase has been exploited for mass rearing the nematode for biological control programs (Bedding 1972). The efficacy of this biological control agent, however, relies in part on its suitability with the *Sirex–Amylostereum* complex, since the virulence of nematode-wasp associations may be specific to certain geographical populations (Bedding 1972). The potential use of *D. siricidicola* in the US is complicated further by the presence of North American *Sirex* species that also carry *Amylostereum* spp. and, thus, could serve as alternate hosts for the introduced nematode. Thirteen *Sirex* species are endemic to the New World and the genus is widespread in North America (Schiff *et al.* 2012). In the northeastern US, three *Sirex* species -*S. cyaneus*, *S. nigricornis*, and *S. nitidus* – have overlapping geographic distributions and host ranges with the introduced *S. noctilio*. Although major host tree preferences vary among the North American woodwasps, all three attack *Pinus* (Schiff *et al.* 2012), and this allows the possibility of host trees with mixed *Sirex* infestations. *S. cyaneus* has been reported to carry only *A. chailletii*, but *S. nigricornis* and *S. nitidus* carry *A. areolatum* or *A. chailletii* (Hajek *et al.* 2013; Olatinwo *et al.* 2014).

As part of our effort to study the biology of the introduced *S. noctilio* and its associated *A. areolatum* genotypes in the US, we determined the genetic diversity of *Amylostereum* spp. associated with *S. noctilio* and other woodwasps from Europe and compared observed genotypes with those from *S. noctilio* collected in the northeastern US.

Materials and methods

Isolate collection and maintenance

Amylostereum samples were obtained from the mycangia of adult female *Sirex* and *Urocerus* spp. either trapped in the field

Please cite this article in press as: Castrillo LA, et al., Multilocus genotyping of *Amylostereum* spp. associated with *Sirex noctilio* and other woodwasps from Europe reveal clonal lineage introduced to the US, Fungal Biology (2015), http://dx.doi.org/10.1016/j.funbio.2015.03.004