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FACTORS INFLUENCING THE CONTROL OF THE SIREX WOODWASP IN SOUTH AFRICA

by

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DECLARATION

I, Brett Phillip Hurley declare that the thesis, which I hereby submit for the degree *Philosophiae Doctor* at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Brett Phillip Hurley

July 2010

I dedicate this thesis to my family. To my wife Tania, my son Joshua and my daughter Mikayla. To my parents, Philip, Linda, Henry and May. To my brothers and sisters, Gareth, Steven, Jonathan, Jim, Shirley and Julie. To my grandmother Joan. And to the rest of my family, both present and future.

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"To praise you is the desire of man, a small piece of your creation. You stir man to take pleasure in praising you, because you have made us for yourself, and our heart is restless until it rests in you." **Confessions, by St Augustine (AD 397)**

SUMMARY

Thesis title: Factors influencing the control of the Sirex woodwasp in South Africa

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The woodwasp *Sirex noctilio* is one of the most serious invasive pests of *Pinus* plantations in the southern hemisphere. Extensive control programs have been developed to manage this pest, of which biological control has been a major component. This thesis examined the factors that could influence the control of *S. noctilio* in South Africa. A critical comparison of *S. noctilio* infestations and control efforts throughout the southern hemisphere revealed that control has not been uniformly effective, and local adaptation of control strategies is likely required as *S. noctilio* moves to new areas. The parasitic nematode *Deladenus siricidicola* is considered the primary biological control agent of *S. noctilio*. This nematode also feeds on the fungal symbiont of *S. noctilio*, *Amylostereum areolatum*. Possible factors influencing the success of this nematode in the summer rainfall areas of South Africa were examined. Data from a field trial revealed that moisture content of the wood influences inoculation success and this is influenced by the time of inoculation and the section of the tree inoculated. Laboratory-based assays revealed that incompatibility between the strain of *A. areolatum* and *D. siricidicola* was unlikely to

be the cause of low inoculation success with the nematode, but that artificial inoculations could be affected by competition of *A. areolatum* with sapstain fungi. The parasitic wasp *Ibalia leucospoides* is another biological agent for *S. noctilio*. Mitochondrial and nuclear DNA sequence data reflected the extensive introduction of *I. leucospoides* into the southern hemisphere, followed by genetic bottlenecks that fixed only a few haplotypes in the introduced populations. Promoting awareness of *S. noctilio* in the forestry community has also been an important component of the control strategy. Data from a survey questionnaire showed that the awareness campaign had been generally successful, but the lack of basic knowledge to identify *S. noctilio* and its symptoms and the poor reach of the awareness media to some sectors of the forestry community, was of concern. This thesis has contributed towards understanding the factors that influence the control of *S. noctilio* in South Africa, with relevance to other regions where *S. noctilio* has been introduced.

PREFACE

Biological invasions can cause considerable losses both to the environment and to the economy of a country. Biological invasions involve the introduction, usually accidental, of a non-native organism, which adapts and establishes in its new environment. In many cases, favourable conditions for the invasive organism, such as the absence of natural enemies, result in an 'unnatural' increase in their numbers. The consequences of such invasions include out competing the native biota and / or drastically reducing the new food resource.

Plantation forestry is particularly vulnerable to biological invasions. This is because plantation forestry often consists of larg stands of exotic trees grown in a monoculture. These plantation trees do well in the absence of their native pests and pathogens. But, when one of these pests or pathogens is introduced, the large expanse of vulnerable hosts, and the absence of natural enemies, makes an ideal environment for their rapid establishment - often rising to epidemic levels.

One of the most serious biological invasions in pine plantation forestry in the past century, particularly in the southern hemisphere, has been the Sirex woodwasp, *Sirex noctilio*. *Sirex noctilio* is native to Eurasia, but has been accidentally introduced to the southern hemisphere, and more recently to North America. In Chapter One of this thesis, we review the introduction and spread of this wasp in the southern hemisphere, and the consequent losses to pine forestry. We also critically evaluate the methods used to control this pest and how successful they have been.

The parasitic nematode, *Deladenus siricidicola*, is regarded as the primary biological control agent against *S. noctilio*. This nematode has been used throughout the southern hemisphere in an effort to manage populations of *S. noctilio*. However, the evaluation of these efforts presented in Chapter One shows clearly that there is variation in the success of this biological control agent. The summer rainfall region of South Africa is an area that has shown particularly poor success with the artificial introduction of *D. siricidicola*. In Chapter Two, I report on an extensive field trial in which possible factors that may influence success with *D. siricidicola* in the summer rainfall areas of South Africa are examined.

Amylostereum areolatum is the fungal symbiont of *S. noctilio*. Together, *A. areolatum* and *S. noctilio* overcome the defence of pine trees, resulting in their death. Besides its mutualistic association with *S. noctilio*, which includes providing nutrition for *S. noctilio* larvae, *A. areolatum* is also a food source for the nematode *D. siricidicola*. In the first two chapters of this thesis the possibility is raised that incompatibility between the strain of *A. areolatum* and the strain of *D. siricidicola*, and / or competition of *A. areolatum* with sapstain fungi in the wood, may influence success with *D. siricidicola* as a biological control agent. In Chapter Three, we describe laboratory based studies to test these two hypotheses. Following on from results obtained in Chapter Two, the effect of water availability on the competitive interactions was also considered in Chapter Three.

Although *D. siricidicola* is considered the primary biological control agent against *S. noctilio*, various parasitic wasps have also been successfully used for this purpose. Of these, *Ibalia leucospoides* has been one of the most successful. This parasitoid wasp has been introduced from its native range in the northern hemisphere to the southern hemisphere. In Chapter Four we examine the introduction history of *I. leucospoides* using mtDNA and nuclear DNA markers. We also compare the diversity between native and introduced populations.

Sirex noctilio has easily been one of the main, if not the main, pest threats to South African pine plantation forestry. Part of the management strategy for *S. noctilio* in South Africa has been to promote awareness of this pest in the forestry community. In Chapter Five we report the use of a survey questionnaire and telephonic interviews to understand the efficacy of these efforts, by examining the perception and knowledge of *S. noctilio* and other forestry pests in the forestry community of South Africa.

Local adaptations to control strategies are often needed as pests move in to new environments. The critical examination of the efforts to control *S. noctilio* in the southern hemisphere (Chapter One) and the results from the field trial (Chapter Two), suggest that such local adaptations to control *S. noctilio* are required in South Africa. Chapter Six of this thesis is a review of the history of *S. noctilio* in South Africa. This includes the current efforts to control the pest as it spreads in the summer rainfall region, where local adaptation to existing control strategies has been required.

The aim of the work represented in this thesis is to contribute to understanding the factors that influence the control of *S. noctilio*. Although the majority of this thesis focused on the southern hemisphere, and more particularly on South Africa, we believe that the findings will have relevance for wherever *S. noctilio* has been introduced. Each of the chapters in this thesis has been written as a potential scientific publication. Thus, some repetition, especially in the introduction of the chapters, was unavoidable.

CHAPTER ONE

A comparison of control results for the alien invasive woodwasp, *Sirex noctilio*, in the southern hemisphere

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Sirex noctilio has resulted in one of the most damaging biological invasions of pine forestry in the southern hemisphere. An intensive, integrated control program has been developed for this pest and is generally considered very successful. However, a critical comparison of *S. noctilio* infestations and control efforts throughout the southern hemisphere reveals that control has not been uniformly effective. Of particular concern is the apparently unexplained variability in establishment and success of biological control agents, including various parasitic wasps and the parasitic nematode, *Deladenus siricidicola*. An overlooked aspect in the establishment of the biological control interventions for *S. noctilio* is the potential influence of genetic bottlenecks on the ability of the agents to adapt to different environments and different populations of *S. noctilio*. Despite established biological control agents, stress in plantations / forests caused by silvicultural practices or the environment will predispose trees to heavy infestation. Unfortunately, improved silvicultural practices are not always economically feasible and environmental changes are often unavoidable. *Sirex noctilio* continues to pose a serious threat to pine forestry in many areas. Despite extensive earlier research into a single integrated control for *S. noctilio*, it is important to recognize that such a strategy will most likely require significant local adaptation in new areas of infestation and over time.

Introduction

Sirex noctilio Fabricius is a woodwasp endemic to Eurasia and northern Africa that infests conifers, mainly species of *Pinus*. Together with its eggs, the female wasp injects toxic mucus and its symbiotic fungus *Amylostereum areolatum* (Chaillet) Boiden into trees (Fig. 1A,B). If the insect becomes successfully established in newly infested trees, the combination of mucus and fungus kills the trees. The larvae develop inside these trees, feeding on fungus-infested wood (Talbot, 1977; Spradbery and Kirk, 1978; Madden, 1988).

Although it is not considered a major pest in its native range, *S. noctilio* has been accidentally introduced to various southern hemisphere countries where it has had a major economic impact on exotic pine plantations (Fig. 1C). The first of these biological invasions was in New Zealand in about 1900, followed by Australia (1952), Uruguay (1980), Argentina (1985), Brazil (1988), South Africa (1994) and Chile (2000) (Miller and Clarke 1935, Gilbert and Miller 1952, Tribe 1995, Maderni 1998, Klasmer *et al.* 1998, Iede *et al.* 1998, Ahumada 2002, Slippers *et al.* 2003) (Fig. 2). Most recently, in 2005, an established population of *S. noctilio* was confirmed in the United States of America (Hoebeke *et al.* 2005).

Slippers *et al.* (2001, 2002) used vegetative compatability groups, DNA sequences and RFLP data of *A. areolatum* to consider the origin and movement of *S. noctilio* in the southern hemisphere. These studies showed that after its introduction to the southern hemisphere, *S. noctilio* most likely spread between the southern hemisphere countries, rather than by new introductions from its native range. It was further shown that *S. noctilio* in Brazil and South Africa share the same origin.

Since its detection in the southern hemisphere, much work has been done in an effort to control populations of *S. noctilio*. For example, in Australia the main strategy for the control of *S. noctilio* following its introduction was to locate and destroy infested trees (Neumann *et al.* 1987, Haugen *et al.* 1990). However, due to the substantial costs associated with this strategy and the realization that *S. noctilio* would not be eradicated from Australia, greater attention was given to biological control. In Tasmania, intensive work on biological control began in 1962, while biological control was adopted as the main strategy in Victoria in 1972 (Taylor 1967, Neumann *et al.* 1987). This approach has subsequently been followed for new *S. noctilio* invasions in other southern hemisphere countries, where biological control has been the main control strategy.

Sirex noctilio primarily attacks stressed trees (Madden 1968). Thus, plantation health plays a crucial role in managing *S. noctilio* populations. Various silvicultural practices have been recommended to minimize the impact of *S. noctilio*. These include pruning trees outside the flight season of *S. noctilio* to avoid stress during this period, timely thinning to reduce competition and the removal of infested trees to eliminate the source for the next season's infestation (Neumann *et al.* 1987). The degree to which these practices have been implemented varies amongst regions and is typically strongly influenced by local economics.

Plantation health is also significantly affected by local environmental conditions. Drought followed by heavy rainfall for a short period can place trees under stress, resulting in their being more prone to attack by *S. noctilio* (Madden 1988). Other conditions that may place trees under stress include fire, winds and excessive dry heat (Madden 1988). Such conditions are generally unpredictable and unavoidable. Thus, control measures have focused on biological control and silvicultural practices.

Control measures for *S. noctilio* invasions have been effective in many regions of the southern hemisphere, but this has not been consistently true. Levels of infestation in South Africa remain variable despite the release of biological control agents. In the Western Cape province, populations of *S. noctilio* remain low, but in parts of the Eastern Cape and KwaZulu-Natal provinces, populations are increasing rapidly, as is the associated tree mortality. Similar variability is also observed between and within other southern hemisphere countries where *S. noctilio* has been introduced (V. Klasmer, pers. comm.).

There have been various reviews of *S. noctilio* and its control in specific regions of the southern hemisphere (e.g., Neumann *et al.* 1987, Iede *et al.* 1998, Tribe and Cillie 2004, Carnegie *et al.* 2005). However, a review of control strategies for this important invasive pest, comparing the situation between these regions, has not been made. This paper considers the spread of *S. noctilio* in southern hemisphere countries and compares the success of efforts to control it. Based on this comparison, hypotheses are presented for possible factors responsible for the variability of control achieved in the various regions. Furthermore, suggestions are made to improve future control of *S. noctilio*, where it has invaded pine plantations and forests outside its native range.

Detection, spread and damage of *Sirex noctilio* in the southern hemisphere

Australasia. *Sirex noctilio* was first reported in the southern hemisphere from standing trees in New Zealand around 1900 (Miller and Clarke 1935). It subsequently spread throughout the country, mainly in *P. radiata* plantations. The pest did not cause serious losses in New Zealand until the drought of 1946. From 1946 to 1951, it was responsible for the devastation of many stands of *Pinus radiata*, killing approximately 20-30 % of intermediate age *P. radiata* in 120 000 ha of unthinned plantations (Rawlings 1955). However, apart from this outbreak, New Zealand has escaped further serious outbreaks of *S. noctilio* (Gilmour 1965, Zondag 1969). This positive situation is believed to be due to climatic conditions favourable for tree growth, major reforms in silvicultural practices to reduce stress and well established populations of biological control agents (Zondag 1969).

In Australia, *S. noctilio* was first detected in Tasmania in 1952 (Gilbert and Miller 1952) and on the mainland of the country in 1961 (Neumann *et al.* 1987). The wasp is presumed to have been accidentally introduced from New Zealand. *Sirex noctilio* is established in Tasmania, Victoria, South Australia, Australian Capital Territory and most of New South Wales, mainly in *P. radiata* plantations (Carnegie *et al.* 2005). It is not yet established in north-eastern New South Wales and has not been detected in Queensland or Western Australia.

Despite considerable investment in research, the steady spread and occasional serious outbreaks of *S. noctilio* were not completely curtailed in Australia (Neumann *et al.* 1987, Madden 1988, Haugen 1990). The ever present, but moderate damage in

Australia was frequently interspersed with serious to very severe outbreaks. The latest of these occurred in the Green Triangle (south-eastern South Australia and south-western Victoria) between 1987 - 1990, despite an established control programme (Haugen 1990). This was primarily due to the absence of monitoring of the *S. noctilio* population, a neglected biological control programme, and overstocked stands (Haugen 1990). This outbreak resulted in the death of approximately 4.8 million trees before 1990 (Haugen and Underdown 1990b, Bedding and Iede 2005). Since then, there have been no serious outbreaks reported from Australia. The majority of infestations in New South Wales from 1996 - 2005 were below 3 %, although mortality has been over 20 % in some areas. The majority of this mortality resulted from unthinned plantations and suppressed trees (Carnegie *et al.* 2005).

<u>South America.</u> In South America, *S. noctilio* was first detected in Uruguay in 1980 (Maderni 1998). Thereafter, it was detected in Argentina in 1985 (Klasmer *et al.* 1998), in Brazil in 1988 (Iede *et al.* 1998) and in Chile in 2000 (Ahumda 2002). It has not yet been detected in other South American countries. Infestations are mainly in *Pinus taeda* and *P. elliottii* plantations in Brazil, northern Argentina and Uruguay, *P. ponderosa*, *P. radiata* and *P. contorta* var. *latifolia* in southern Argentina, and *P. radiata* plantations in Chile (Maderni 1998, Ahumada 2002, Carnegie *et al.* 2006, V. Klasmer, pers. comm.).

Over the 20 years since its introduction into South America, *S. noctilio* damage has varied from very minimal to devastating in some areas. This is despite widespread awareness of its potential impact and attempts to control it. Tree mortality has been over 60 % in some stands in Argentina (V. Klasmer, pers. comm.) and as high as 70 % in some stands in Uruguay (Maderni 1998). In Brazil, 350 000 ha of pine plantations are infested and an estimated US$ 6.6 million would be lost each year if an integrated pest management programme were not in place (Bedding and Iede 2005). Large areas of *P. radiata* in Chile are susceptible to infestation, but populations of *S. noctilio* are currently low in that area and they are not widespread (R. Ahumada, pers. comm.).

<u>Africa.</u> *Sirex noctilio* was first reported in imported wood in South Africa at a timber yard in Port Elizabeth in 1962 (Taylor 1962). At that time the wasp apparently did not escape to become established in the pine plantations of South Africa. In April 1994, *S. noctilio* was reported in Cape Town from *P. radiata* plantations (Tribe 1995). During the first three seasons after its initial discovery, the wasp spread in a 90 km arc through pine plantations of this region (Tribe and Cillié 2004). In 2002, *S. noctilio* was detected in the Eastern Cape and KwaZulu-Natal provinces (authors unpublished). A variety of *Pinus* species were infested, but mostly *P. radiata* in the Western Cape and *P. patula* in the Eastern Cape and KwaZulu-Natal. Of the remaining provinces, Mpumalanga and the North Province are the most seriously threatened by *S. noctilio*. This is because approximately 50 % of pine plantations in South Africa are found in these two provinces (Anon 2005).

Sirex noctilio infestation levels in the Western Cape province of South Africa have generally been low. The main exception was an infestation in George in 2002, where tree mortality was an average of 10 % in 100 ha of 12 - 13 year-old *P. radiata* (M. Strydom, pers. comm.). These trees were overstocked and the infestation subsided during the course of the following year. Infestation levels in the north Eastern Cape and

KwaZulu-Natal were substantially higher than those reported in the Western Cape, with a number of compartments having over 10 % infestation, and some higher than 35 % (P. Croft, pers. comm.). Currently, it is estimated that approximately 35 000 ha of pine in the Eastern Cape and KwaZulu-Natal are infested to a mean level of 6 %, with a total estimated value of damage being R300 million per annum (M.J. Wingfield, unpublished).

Biological control in the southern hemisphere

Parasitic wasps

Australasia. From 1928 - 1968, 11 species of parasitic wasps were received from the USA, Europe and Asia for rearing in New Zealand (Nuttall 1989). Of these, only five species were eventually released in that country (Table 1). The first of these was *Rhyssa persuasoria persuasoria* (L.), collected in England, and introduced into New Zealand from 1928 (Hanson 1939, Nuttall 1989). This represented the first attempt to control *S. noctilio* as an alien invasive pest using a biological control agent. Shortly after this, attempts were made to establish the European parasitic wasp, *Ibalia leucospoides leucospoides* (Hockenwarth). Initial attempts were unsuccessful, but by 1954 numerous releases of *I. l. leucospoides* had been made in New Zealand and it was reported to be well established by 1957 (Zondag 1969). The other parasitic wasps released in New Zealand were *Megarhyssa nortoni nortoni* (Cresson) from the USA, *Rhyssa persuasoria himalayensis* Wilkinson from Pakistan and India, and *Ibalia leucospoides ensiger* Norton, originally from the U.S.A. but reared and released from Tasmania (Taylor 1967, Nuttall, 1989). Of these five parasitic wasps released in New Zealand, all but *R. p. himalayensis*, became well established (Table 1). Parasitism by *I. l. leucospoides* was recorded between 25-35 % on average, but sometimes as high as 55 %, while the combined parasitism of *I. l. leucospoides* and the rhyssines was over 70 % in some areas (Nuttall 1989). Interbreeding is known to occur between *I. l. leucospoides* and *I. l. ensiger* and between *R. p. persuasoria* and *R. p. himalayensis*, with the hybrids being indistinguishable from *I. l. leucospoides* and *R. p. persuasoria* respectively (Nuttall 1989). Thus, it is probable that releases of *I. l. leucospoides* and *R. p. persuasoria* in New Zealand and the rest of the southern hemisphere have also included these hybrids.

Nine species of parasitic wasps were released into Australia as biological control agents from the late 1950's (Table 1). Of these, *I. leucospoides*, *M. nortoni* and *R. persuasoria* have been the most successful. Taylor (1978) showed that *M. nortoni* and *R. persuasoria* were responsible for reducing *S. noctilio* populations in Tasmania between 1965 and 1974. *Ibalia leucospoides* was considered the most effective parasitic wasp in Victoria and New South Wales, attaining up to 40 % parasitism in some areas of Victoria (Neumann *et al.* 1987, Carnegie *et al.* 2005). In combination, the named parasitic wasps usually do not kill more than 40 % of a *S. noctilio* population and are, therefore, not considered sufficient to control *S. noctilio* on their own.

South America. The parasitic wasp *I. leucospoides* was first recorded in Uruguay in 1984, where it had apparently been introduced naturally with its host *S. noctilio* or another siricid, *Urocerus gigas.* It has subsequently spread with the pest complex throughout Uruguay, Argentina, Brazil and Chile (Eskiviski *et al.* 2004; R. Ahumada, pers. comm.).

The natural migration, and occasional human assisted introductions, of *I. leucospoides* in South America has resulted in considerable, although variable parasitism. Iede *et al.* (2000) reported parasitism rates of *I. leucospoides* in Brazil to be as high as 39 %, and 25 % on average, which correlates well with the parasitism rates obtained in Australasia. Similarly, parasitism in the Andean Patagonian region of Argentina ranged from 20 % to 40 % (Klasmer 1998). Greater variation in parasitism was found between three sites in Misiones, Argentina, where parasitism was 0 %, 2.4 % and 35 % in the three sites respectively (Eskiviski *et al.* 2004). Eskiviski *et al.* (2004) suggested that this variation was due to differences in the age of the trees at the different sites, where *S. noctilio*, and therefore *I. leucospoides*, would be more recently established in sites of younger trees. Limited information is, however, available on the first appearance or releases of *I. leucospoides* at these various sites, which make direct comparisons between these reported parasitism rates difficult.

Rhyssa persuasoria and *M. nortoni* were imported and released in many affected areas of South America. The first introduction of *M. nortoni* was with wasps sent from Tasmania to Brazil in 1996 (Iede *et al.* 2000). Fifty females and 27 males were used to start a rearing colony. The first release was in 1997 with only 18 females, but 136 males and 97 females were released the following year. *Rhyssa persuasoria* was first introduced in Brazil in 1997 (Iede *et al.* 2000). Only nine females were used to start a rearing colony, resulting in the release of only two males and 10 females the following year. Information on the establishment and further releases of *I. leucospoides*, *R. persuasoria* and *M. nortoni* within South America is not available.

Africa. Of the various parasitic wasps used to control *S. noctilio*, only *I. leucospoides* and *M. nortoni* have been released in South Africa. Of these, only *I. leucospoides* is known to have become established (Table 1). Eighteen females and 19 males of *I. leucospoides* were imported from Uruguay and reared in captivity for subsequent releases (Tribe and Cillié 2004). Thus, these releases were of very limited diversity and the source population (Uruguay) was from an unknown origin. Despite these limited releases (Table 1), establishment of this parasitic wasp has been confirmed in some plantations of the Western Cape. Sirex-infested logs collected in that area from 2001-2005 reveal that *I. leucospoides* has become established in some areas, but not in others (authors unpublished). It is unknown whether the 176 *I. leucospoides* collected from the Western Cape in 2006 and released the same year in the Eastern Cape and KwaZulu-Natal, have become established. Forty-four mated females and ten males of *M. nortoni* were brought from Tasmania to South Africa in 1998 and reared in captivity (Tribe and Cillié 2004). A very small number of *M. nortoni* were released the following year in an isolated pine stand (Table 1). *Megarhyssa nortoni* were not found in Sirex-infested logs collected from the original release stand in 2003, nor has *M. nortoni* been recovered from the field in nearby plantations during surveys from 2001 – 2003.

Factors influencing successful establishment of parasitic wasps. It is clear that parasitic wasps play an important, albeit not primary role in the control of *S. noctilio* in the southern hemisphere. *Ibalia leucospoides*, *M. nortoni* and *R. persuasoria* are generally considered to be the most important parasitic wasps of *S. noctilio* and in combination they can achieve significant levels of parasitism. Data pertaining to the release and establishment of these parasitic wasps in the southern hemisphere is incomplete, but it is evident that great variation in establishment exists between regions. In South Africa in particular, no parasitic wasps are currently well established. The establishment of *I. leucospoides*, *M. nortoni* and *R. persuasoria* is especially needed in KwaZulu-Natal, where *S. noctilio* is currently in an epidemic phase.

The population variation of the parasitic wasps within and between different regions is not well understood. Releases of *Rhyssa* spp. in Tasmania and New South Wales, releases of *R. persuasoria* and *M. nortoni* in parts of South America, and releases of *I. leucospoides* in South Africa, have been from very small numbers of wasps (Table 1). Such small releases of biological control agents have been known to result in genetic bottlenecks (Hufbauer *et al*. 2004, Lloyd *et al.* 2005). Although the exact influence of genetic bottlenecks on the success of biological control agents is unclear, low genetic diversity could decrease the ability of the biological control organism to adapt to new environments and host types (Baker *et al.* 2003, Roderick and Navajas 2003, Lloyd *et al.* 2005).

Villacide and Corley (2003) showed a good match between the climate of Argentina and the species characteristics of *I. leucospoides* using CLIMEX. However, the species characteristics used are from its native environment, where genetic diversity is expected to be high, and does not necessarily reflect introduced populations of *I. leucospoides*, where genetic diversity is low. Thus, research is needed to determine the adaptability of parasitic wasp populations to new environments as influenced by their genetic diversity. Other important factors that could influence the establishment of these parasitic wasps include the population density of *S. noctilio* when the parasitic wasps are released, the ratio of female to male wasps released, and the synchrony between the parasitic wasps and *S. noctilio* life cycles, as *I. leucospoides* only parasitises eggs and first instar larvae of *S. noctilio*.

Parasitic nematodes

Australasia. In 1962, the nematode *Deladenus* (*Beddingia*) *siricidicola* Bedding was found infecting *S. noctilio* in New Zealand, on *P. patula* logs in the North Island (Zondag 1969). These nematodes entered New Zealand together with *S. noctilio* and were not intentionally introduced from Eurasia, where the wasp is native. Subsequent surveys showed that the nematode was present in most Sirex-infested plantations on the North Island, with some plantations having infection levels of *S. noctilio* as high as 90 % (Zondag 1969, 1979). No nematodes were found on the South Island. In 1967, Zondag (1971) tested various methods to artificially introduce the nematode from the North Island to the South Island. This resulted in nematodes being introduced into the South Island by means of moistened wood shavings from 1969 to 1970 (Zondag 1979). From 1971 onwards, the gelatin-based method (Bedding and Akhurst 1974) was used. Approximately 200 trees in the South Island were inoculated with the nematode from 1967 to 1974. By 1975 infections of *S. noctilio* of over 75 % were recorded from some areas of the South Island from uninoculated trees (naturally introduced nematodes) (Zondag 1979). No further active releases of the nematode were made after the 1970s (J. Bain, pers. comm.).

Hundreds of isolates of seven species of *Deladenus* that were found parasitizing siricids in their native range were collected and screened for selectivity and high levels of parasitism (Bedding and Akhurst 1978, Bedding and Iede 2005). This resulted in the selection of a strain of *D. siricidicola* from Sopron, Hungary, known as the Sopron strain. Infections by this *D. siricidicola* strain were raised to levels of almost 100 % in inoculated trees in Australia (Bedding and Akhurst 1974). From the first experimental liberation of *D. siricidicola* (Sopron strain) in 1970 in northern Tasmania, 92 % of Sirex-infested trees from a 12 ha compartment contained nematodes in just two years, where an estimated 50 parasitised *S. noctilio* wasps emerged from inoculated logs (Bedding and Akhurst 1974).

A loss of virulence of *D. siricidicola* was first detected during the Green Triangle outbreak in 1987 - 1990 (Haugen and Underdown 1993). This loss in virulence resulted from rearing the nematode in laboratory cultures for over 20 years without allowing it to go through a parasitic cycle (Bedding and Iede 2005). To establish new cultures for laboratory breeding, nematodes were recollected during 1991 from the Kamona forest in Tasmania (the site of the first nematode liberations in Australia during 1970). This 'Kamona' strain was subsequently reared and released to replace the Sopron strain throughout Australia.

South America. The depth of experience on the biological control of *S. noctilio* resulting from the Australia and New Zealand situation, contributed to the establishment of biological control programmes in South America soon after the detection of the pest in this region. The Sopron strain of *D. siricidicola* was imported into Brazil from Australia in 1989, with the first inoculations in Brazil occurring that same year (Iede *et al.* 2000). The Kamona strain was later imported in 1995 (Bedding and Iede 2005). Nematodes were isolated from infected insects in Encruzilhada do Sol, Brazil, in 1995, and these were used to establish a laboratory colony in Misiones, Argentina (Eskiviski *et al.* 2003). Likewise, Uruguay and Chile have also imported the nematode from Brazil for direct inoculations or to establish a laboratory colony (R. Ahumada, pers. comm.).

Establishment of *D. siricidicola* in South America has been variable across different areas. Parasitism from uninoculated trees have been up to 70-80 % parasitism from a 12000 ha *P. taeda* plantation in the Rio Grande do Sul state in Brazil (Iede *et al.* 1998), and 85 % from the Andean Patagonian region of Argentina (V. Klasmer, pers. comm.). Despite these successful cases, parasitism from inoculated trees has often been very low. In the Santa Cantarina state of Brazil, only 18.84 % parasitism was obtained from directly inoculated logs (n = 1810 wasps) (Fenili *et al.* 2000). Fenili *et al.* (2000) suggested loss of nematode viability or virulence, inoculation technique, nematode migration ability, and climatic conditions, as possible reasons for the low level of parasitism. In Argentina, Eksiviski *et al.* (2003) compared parasitism rates between nematodes originally obtained from Brazil and reared for 3 years and nematodes obtained from infected wasps caught in Misiones and reared for one year. From the two sites tested, parasitism from inoculated logs was 2 % and 35 % (n = 60 wasps) for the three-year and one-year source respectively at the one site, and 10.3 % and 15.5 % (n = 201 wasps) for the three-year and one-year source respectively at the other site tested. Similarly, Becerra *et al.* (2000) reported parasitism below 5 % from trees inoculated in some areas of Misiones. Data were unavailable on whether the nematode has managed to establish in these areas despite the low inoculation success, but the difference in inoculation success in these areas, compared to the almost 100 % obtained in Australia (Bedding and Akhurst 1974), is reason for concern.

Africa. Similar to the situation in South America, South Africa has benefited considerably from experience regarding the biological control of *S. noctilio* in Australasia. It was thus possible to introduce biological control agents soon after the detection of *S. noctilio* in the Western Cape. The Kamona strain of *D. siricidicola* was the first biological control agent introduced into South Africa. During 1995 and 1996, 50 and 20 million nematodes respectively were used to inoculate 400 trees in a 90 km arc around Cape Town. Parasitism rates in uninoculated trees were assessed in one of the plantations inoculated in 2005 and found to be 22.6 % in 1996 (n = 402 wasps), 54 % in 1997 (n = 89 wasps) and 96.1 % in 1998 (n = 77 wasps) (Tribe and Cillié 2004). These sample sizes are small and might not have represented the population as a whole. Logs collected in 2001 to 2002 from six of the plantations inoculated in 1995/6 showed variable parasitism ranging from 0 % to 64 %, with an average of 20 % (n = 191 wasps). Logs collected in this period from six plantations in the Western Cape that were not inoculated in 1995/6 showed no parasitism (n = 162 wasps) (authors unpublished). For logs collected in 2005 from previously inoculated plantations, percentage parasitism varied from 0-30 % (n = 168 wasps) (authors unpublished). Although these results are from a very limited sample, they do show variable levels of parasitism in the Western Cape, even for areas that had previously been inoculated with the nematode. It is not known if the variation in parasitism is a consequence of the low population of *S. noctilio* in this region or due to the failure of the nematode to establish.

Between 1996 and 2003, no further nematodes were released for the biological control of *S. noctilio* in South Africa. Shortly after the serious new invasion of this pest in 2003, 180 million nematodes were used to inoculate approximately 1800 trees in 2004. These inoculations were mainly in the Eastern Cape and KwaZulu-Natal, where infestations of *S. noctilio* were highest. This inoculation programme was increased in 2005, with approximately 480 million nematodes used to inoculate about 4400 trees. These nematodes were of the Kamona strain imported from Australia, but reared in South Africa. Both these inoculations gave poor results, with less than 5 % parasitism obtained from trees inoculated in 2004 (n = 2472 wasps) and less than 10 % parasitism obtained from trees inoculated in 2005 (n = 13999 wasps) (authors unpublished). These disappointing results were despite considerable efforts to streamline rearing, transport and inoculation methods for the nematodes used in the 2005 inoculations. Parasitism in naturally infested trees has yet to be determined in these areas.

Factors influencing successful establishment of *D. siricidicola.* Although *D. siricidicola* inoculations have been a success in most areas where they have been applied, it is clear this is not uniformly true for all areas. Even within countries, parasitism in inoculated trees has been variable, ranging from 0 % to nearly 100 %. Various factors could influence the inoculation success and therefore the efficacy of *D. siricidicola* as a biological control agent. Possible factors include inoculation technique, moisture content of the wood, loss of virulence in the nematode, incompatibility among the specific populations of *S. noctilio*, *A. areolatum*, and *D. siricidicola* in that area, and pine species.

1. Inoculation technique. One of the important factors that can influence the efficacy of *D. siricidicola* as a biological control agent is the technique used to inoculate trees with the nematode. Bedding and Iede (2005) describe the inoculation technique in detail (see Fig. 1D-G). Inappropriate application of this technique, including the use of blunt inoculation hammers, is known to result in a drastic reduction in inoculation success (Bedding and Akhurst, 1974, Bedding and Iede 2005). After the poor parasitism results obtained in South Africa for the 2004 inoculations, special attention was paid to inoculation techniques, as it was thought that this could have resulted in the poor results. Despite these refinements, parasitism results the following year remained low. Similarly, in Argentina, the same inoculation technique was applied in Misiones and the Andean Patagonian regions and yet parasitism results are high in the Andean Patagonian region and low in Misiones (V. Klasmer, pers. comm.). Thus, though inoculation technique can influence the success obtained with the nematode, it can be easily addressed and is not believed to be the primary factor explaining low success in South Africa and parts of South America.

2. Moisture content. Moisture content of the wood during the period for nematode infection of *S. noctilio* larvae can be a key factor explaining the variation in inoculation success. Bedding and Akhurst (1974) indicated that *D. siricidicola* prefers moisture content of the wood to be 50 % and higher for successful establishment. In contrast, Haugen and Underdown (1993) concluded that moisture content was not a major factor causing low levels of parasitism in *P. radiata* logs inoculated in Australia. Moisture content of these logs had ranged from 33 % to 72 %, with a mean of about 45 %. However, preliminary studies in KwaZulu-Natal indicate that moisture content is as low as 15 % in the top section of trees during inoculation, far lower than recommended by Bedding and Akhurst (1974) or tested by Haugen and Underdown (1993). Inoculations in KwaZulu-Natal are done from March to July, and wasps begin to emerge in October. As KwaZulu-Natal is a summer rainfall area, the nematodes are in the wood during the dry season. This is in contrast to the Western Cape, where most inoculations are done from June to July, and wasps begin to emerge in November. The Western Cape is a winter rainfall area, thus the nematodes are in the wood during the wet season. Areas of New Zealand and Australia where *S. noctilio* occurs and *D. siricidicola* is successfully applied are also mainly winter or all year rainfall areas. Details of the rainfall and moisture content of trees in Sirex-infested areas of South America are not currently available. Nonetheless, there is good evidence to suggest that moisture content in trees could have influenced poor levels of nematode parasitism in some areas.

Further studies are underway to consider the influence of moisture content on nematode establishment and survival in the KwaZulu-Natal province of South Africa. In this regard, it will be particularly valuable to have a comparison of parasitism in this area, and the Western Cape province, where moisture content in the trees could differ due to the different pine species planted and the different climates. If the influence of climate on moisture content is a serious barrier to nematode movement and survival in certain areas, the technique to release *D. siricidicola*, and possibly the feasibility of using this agent in these areas at all will need to be re-examined.

Parasitism rates with *D. siricidicola* are still relatively low in the Western Cape province of South Africa. This is despite the fact that the area has a climate similar to that of New Zealand and Sirex-infested areas of Australia. The variable parasitism rates in the Western Cape may not be a result of the nematode's inability to establish in these areas, as observed by the initial inoculations in this area, where high parasitism was obtained (Tribe and Cillie 2004). Rather, the limited initial inoculations in the Western Cape and the small *S. noctilio* population may be the cause of the current low parasitism levels.

3. Loss of virulence. The loss of virulence in *D. siricidicola* can be an important factor influencing the success of this biological control agent. *Deladenus siricidicola* could loose its ability to change to the parasitic form when reared in the laboratory for long periods, as was the case in the Green Triangle (Haugen and Underdown 1993, Bedding and Iede 2005). To overcome this potential obstacle, nematodes are stored in liquid nitrogen and only reared for a short period in culture before release in the field. In the Western Cape province of South Africa, nematodes stored in liquid nitrogen cultures in Australia were directly inoculated into trees. In KwaZulu-Natal, nematodes were imported from Australia and then further reared in South Africa before release. Nematodes were reared for three months after arrival from Australia, before they were released in the field for the 2004 inoculations in KwaZulu-Natal. In the case of 2005 inoculations, they were reared for about 15 months before release although they were also stored at 5˚C for brief periods in this time. It is possible that nematodes released in 2005 may have had reduced virulence but this could not have been the case for those released in 2004. Yet results from both years' inoculations were low. In Argentina, the nematodes released in Misiones where parasitism is low and in the Andean Patagonian region where parasitism is high, are both produced in the same facility in Misiones (V. Klasmer, pers. comm.). One would thus not expect differences in virulence. Thus, at least in some cases, loss of virulence is unlikely to be responsible for the low parasitism achieved.

4. Incompatibility between populations. Incompatibility between specific nematode and wasp strains can be a significant barrier to the use of *D. siricidicola*. Bedding (1972) showed that different populations of *S. noctilio* were differently affected by the same nematode strain. In certain strains of *S. noctilio,* the nematodes are released far too late to penetrate the wasp eggs, as the egg shells have already formed (Bedding and Iede 2005). Despite its importance, the presence of different strains of *S. noctilio* within and between southern hemisphere countries has not been examined.

All strains of *D. siricidicola* are not equally compatible with all strains of *A. areolatum*. Certain isolates from the field in Australia were found to be better for rearing the nematode than others (R.A. Bedding, pers. comm., in Slippers *et al.* (2001)). Similarly, in South Africa, preliminary observations reveal that nematodes imported from Australia are more easily reared using the fungus imported with the nematodes than using the fungus isolated from the field in KwaZulu-Natal and Western Cape. Incompatibility between the nematode and fungus would influence the feeding and reproduction of the nematode on the fungus. This in turn would affect the survival and spread of the nematode in the tree and its potential to parasitise *S. noctilio* larvae.

A serious shortcoming in our understanding of the biological control of *S. noctilio* is the complete absence of any information regarding population variation and structure of *D. siricidicola* in the southern hemisphere. The majority of *D. siricidicola* releases in the southern hemisphere have been with the Kamona strain. Thus, the genetic and phenotypic diversity of *D. siricidicola* in the southern hemisphere and its ability to adapt to different conditions could be limited. Given specific interactions of this nematode with *S. noctilio*, *A. areolatum*, and possibly the environment, this area of research requires urgent attention.

5. Pine species. Pine species differ across the different regions affected by *S. noctilio*, and include *P. radiata*, *P. patula*, *P. taeda*, *P. carribea*, *P. pondersae*, *P. elliottii*, *P. contorta* var. *latifolia*, and others. Differences between these species, such as resin composition, tracheid structure, moisture content and other factors could influence nematode establishment. The influence of these factors is currently unknown and there is a clear need for future research to address these questions.

Silvicultural control in the southern hemisphere

Poor silviculture and environmental events, leading to stress on trees, have been a key factor in most major outbreaks in Australia and New Zealand (Madden 1988). That stressed trees are more susceptible to attack by *S. noctilio* is well known (Madden 1968, Talbot 1977, Neumann and Minko 1981, and others). *Sirex noctilio* females test the vigour of trees with their ovipositors, thus determining the osmotic pressure. High osmotic pressure is found in trees with high levels of vigour, and these trees are generally rejected by female wasps, while trees with low osmotic pressure are generally more susceptible to attack (Madden 1968). Stress in trees may result from: 1. suppression, for example in overstocked stands where competition is high; 2. physical damage, including damage that occurs during pruning; 3. attack by insect or disease; 4. unfavourable environmental conditions; and other factors.

Silvicultural practices, as described by Haugen *et al.* (1990), Neumann *et al.* (1987) and others, have been a key component to control *S. noctilio* in Australia and New Zealand. After the outbreak of *S. noctilio* in New Zealand between 1946 to 1951, improvements in silviculture, together with the introduction of parasites, kept *S. noctilio* populations low (Neumann and Minko 1981). The importance of silviculture for control in Australia was emphasized by Neumann *et al.* (1987) who stated that outbreaks were largely a management problem that could be prevented by routine surveillance of plantations and the application of silviculture measures. Neumann *et al.* (1987) further stated that biological control measures were not necessary for some well-managed plantations. Likewise, the majority of recent mortality in New South Wales and Tasmania above 3 % was associated with unthinned stands, stands with suppressed trees or summer pruning (Carnegie *et al.* 2005).

Silvicultural practices differ between and within southern hemisphere countries (Table 2). There is no great difference in initial stand density of *Pinus* species within the southern hemisphere. KwaZulu-Natal, South Africa is the only region where no thinning occurs before harvest. As a result, stand density may be as high as 1250 spha at time of harvest. In contrast, stand density at time of harvest generally does not exceed 500 spha in other Sirex-infested regions of the southern hemisphere. Although stand density is also high in other regions before the first thinnings, the trees are generally too small at this stage to be favourable to *S. noctilio* infestation. High stand density is known to increase the stress on trees, which predisposes them to attack by *S. noctilio* (Neumann *et al.* 1987).

Pinus plantations in KwaZulu-Natal provide an abundance of stressed trees, and together with a lack of established biological control agents, *S. noctilio* is currently causing substantial damage in this region.

Despite the importance of silvicultural measures, market demands and difficult terrain can result in situations, such as delayed thinning, which favour a build-up of *S. noctilio* populations (Carnegie *et al.* 2005). In areas where the main market for timber is pulp, and the objective is to obtain as much wood volume as possible at time of harvest, stand density is typically very high. Many Sirex-infested areas of the southern hemisphere represent plantations where sawn-timber is the main product. Thus, individual tree size and timber quality is important and sites are thinned to reduce competition. To change the management styles of a pulp regime, for example to include thinning, could seriously affect profitability of plantations and in some cases this may not be economically viable.

Other control methods in the southern hemisphere

Other important management considerations include eradication and quarantine. Eradication is not a feasible option where *S. noctilio* has become established, but destruction of infested logs can be important in maintaining a low population of *S. noctilio* in newly infested areas. In the Western Cape province of South Africa, all infested trees that could be found were removed and burned immediately after detection of *S. noctilio*, and older (>40 years) infested compartments were clear felled (Tribe and Cillié 2004). These measures most likely had a significant impact on lowering the initial populations and slowing the population build-up of *S. noctilio*. In KwaZulu-Natal, harvesting of severely infested compartments and the processing and burning of this timber began in 2005. Thus, the opportunity to impair the spread and population build-up of *S. noctilio* was most likely not achieved.

Quarantine measures that prevent the movement of timber from infested areas to non-infested areas are essential. In Australia, quarantine measures restrict the movement of Sirex-infested pine into the states of Queensland and Western Australia (Carnegie *et al.* 2006). In South Africa, established populations of *S. noctilio* have only been detected in the Western Cape, Eastern Cape and KwaZulu-Natal provinces. These populations are greater than 200 km from the closest pine plantations in the Mpumalanga province. It would thus take an estimated five years for *S. noctilio* to arrive in this uninfested area, assuming a movement of 40 km per year, as observed by Eldridge and Taylor (1989). It is therefore essential to establish strict quarantine on the movement of infested timber so that *S. noctilio* does not move more rapidly into uninfested areas, than it will do naturally. Likewise, quarantine is also needed in South America to prevent the movement of *S. noctilio* to countries where pines are widely grown and where *S. noctilio* has not yet appeared (e.g. Equador, Colombia and Venezuela). Quarantine should also aim to limit further international movement of *S. noctilio*, which could result in the introduction of different genotypes of *S. noctilio* and / or *A. areolatum*.

Discussion

Since the first arrival of *S. noctilio* in the southern hemisphere, this alien invasive pest has resulted in severe losses to pine forestry in every country where it has become established. In all of these countries, control programmes have been established to counter increasing *S. noctilio* populations. The success of these control programmes, including both silvicultural control measures and biological control, has been variable. In New Zealand, *S. noctilio* is no longer considered a major threat and an active control programme is not considered necessary. In Australia, infestations are mostly below 1 %, although an active control programme remains in place. *Sirex noctilio* is still considered a major threat in South America, where biological control has been very successful in some areas, but less so in others. In South Africa, infestations remain low in the Western Cape, but are above 30 % in some areas of KwaZulu-Natal and the Eastern Cape, and they are increasing in these provinces.

The nematode *D. siricidicola* and various parasitic wasp species have been introduced as biological control agents in all southern hemisphere countries where *S. noctilio* has been introduced. Amongst these agents, *D. siricidicola* has been considered the primary biological control tool, and it has been particularly successful in Australia. However, inoculation success with this nematode has been variable in South America and South Africa, ranging from very poor to good. Preliminary evidence from current assessments also suggests that this low introduction success, translates into poorer establishment and spread of *D. siricidicola* than is seen in areas with high initial introduction success. The long term influence of low inoculation success in these environments needs to be determined. It is also crucial that the causal factors resulting in low inoculation success and parasitism are discovered and resolved. These causal factors might be unavoidable, for example low moisture content of the wood or incompatibility between strain of nematode and wasp or nematode and fungus. In such cases, the feasibility of using *D. siricidicola* will need to be re-examined and further species or strains of *Deladenus* might need to be evaluated to match specific conditions.

In addition to variation in the establishment of *D. siricidicola*, the establishment of parasitic wasps is also not consistent throughout the southern hemisphere. This is especially true regarding South Africa, where only *I. leucospoides* is established in parts of the Western Cape. Further introductions of *I. leucospoides* and introductions of *M. nortoni* and *R. persuasoria* are needed in these areas. These introductions should entail large numbers of wasps to avoid possible genetic bottlenecks associated with releases of small numbers as illustrated by Roderick and Navajas (2003).

The common denominator for outbreaks of *S. noctilio* in the southern hemisphere has been an abundant supply of stressed trees. This has primarily been due to environmental stress and / or associated silvicultural practices, especially heavily stocked stands. In places where such adverse conditions are perpetuated, such as parts of Australia and South America and the Eastern Cape and KwaZulu-Natal province of South Africa, *S. noctilio* remains a substantial threat where high infestations of the wasp can occur, even in the presence of an active biological control programme. The importance of silviculture is evident, notably in South Africa, where differences in the planting regimes in the Western Cape and the north Eastern Cape / KwaZulu-Natal areas is most likely an important reason for the difference in infestations in these areas. Without silvicultural adjustments in these areas, high infestations are likely to continue, even in the presence of an established biological control programme

A selective reading of the literature pertaining to this pest, could easily promote the view that *S. noctilio* is a serious pest, but one that is easily controlled. While extensive research has been undertaken to develop a control strategy that is effective in many areas, it is important to recognize that this strategy might require significant local adaptation. This is especially regarding the application of biological control agents. The interactions between *S. noctilio*, its fungal symbiont *A. areolatum*, biological control agents and the environment are still poorly understood. This hinders local adaptations to control programmes in areas where these are seriously needed. Great opportunities exist to study the interactions among these organisms using modern ecological and molecular tools. Such studies are likely to significantly advance our knowledge of the very complex interactions that typify *S. noctilio* infestations in introduced environments.

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Table 1. Parasitoid wasps of *Sirex noctilio* released in the southern hemisphere[a].

	Rhyssa persuasoria persuasoria				*Rhyssa persausoria himalayensis*				*Rhyssa hoferi*				*Rhyssa lineolata*			
	Years liberated	Male	Female	First recovered from field	Years liberated	Male	Female	First recovered from field	Years liberated	Male	Female	First recovered from field	Years liberated	Male	Female	First recovered from field
New Zealand	1929-1950	52[b]	3500	1936	1964-1970	89	198	not recovered	_	_	_	_	_	_	_	_
Australia - Tasmania[c]	1957-1965	0	56	1959	1964	111	128	not recovered	_	_	_	_	1962-1965	452	53	1963
Australia - Victoria	1970-1985	2852	3685	1988	_	_	_	_	1980-1982	not known	32	1988	_	_	_	1988
Australia - New South Wales	1980-1983	256[d]		not recovered	_	_	_	_	1982-1984	78[d]		1988/1989	_	_	_	_
South Africa - Western Cape	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
South Africa - KwaZulu-Natal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

	Ibalia leucospoides leucospoides				*Ibalia leucospoides ensiger*				*Schlettererius cinctipes*				*Megarhyssa nortoni nortoni*			
	Years liberated	Male	Female	First recovered from field	Years liberated	Male	Female	First recovered from field	Years liberated	Male	Female	First recovered from field	Years liberated	Male	Female	First recovered from field
New Zealand	1954-1978	8537	8096	1957	1966-1971	1883	1729	1968	_	_	_	_	1964-1984	1258	1863	1968
Australia - Tasmania[c]	1959-1966	2015	2005	1960	1965-1966	297	236	1966		not known		1973	1965-1966	138	301	1966
Australia - Victoria	1967?-1985[e]	89033+	79084+	1969?	[e]	[e]	[e]	[e]	1970-1985	317	1095	1988	1970-1985[f]	10903	12494	1972
Australia - New South Wales	1980-1989	19331[d]		1991/1992	_	_	_	_	1981-1983	297[d]		early 1980s	1980-1989	4087[d]		1991/1992
South Africa - Western Cape	1998-2001	456[d]		2002	_	_	_	_	_	_	_	_	1999	41	38	not recovered
South Africa - KwaZulu-Natal and Eastern Cape	2006	176[d]		not yet attempted	_	_	_	_	_	_	_	_	_	_	_	_

[a] data from Taylor (1967), Taylor (1978), Neumann & Minko (1981), Neumann *et al.* (1987), Nuttall (1989), Haugen & Underdown (1990a), Tribe & Cillie (2004), Carnegie *et al.* (2005), and authors unpublished.

[b] male numbers for 1929-1932 only.

[c] male and female numbers for 1962-1967 only.

[d] total male and female wasps.

[e] releases contained both *I. l. leucospoides* and *I. l. ensiger.*

[f] releases contained both *M. n. nortoni* and *M. n. quebecensis.*

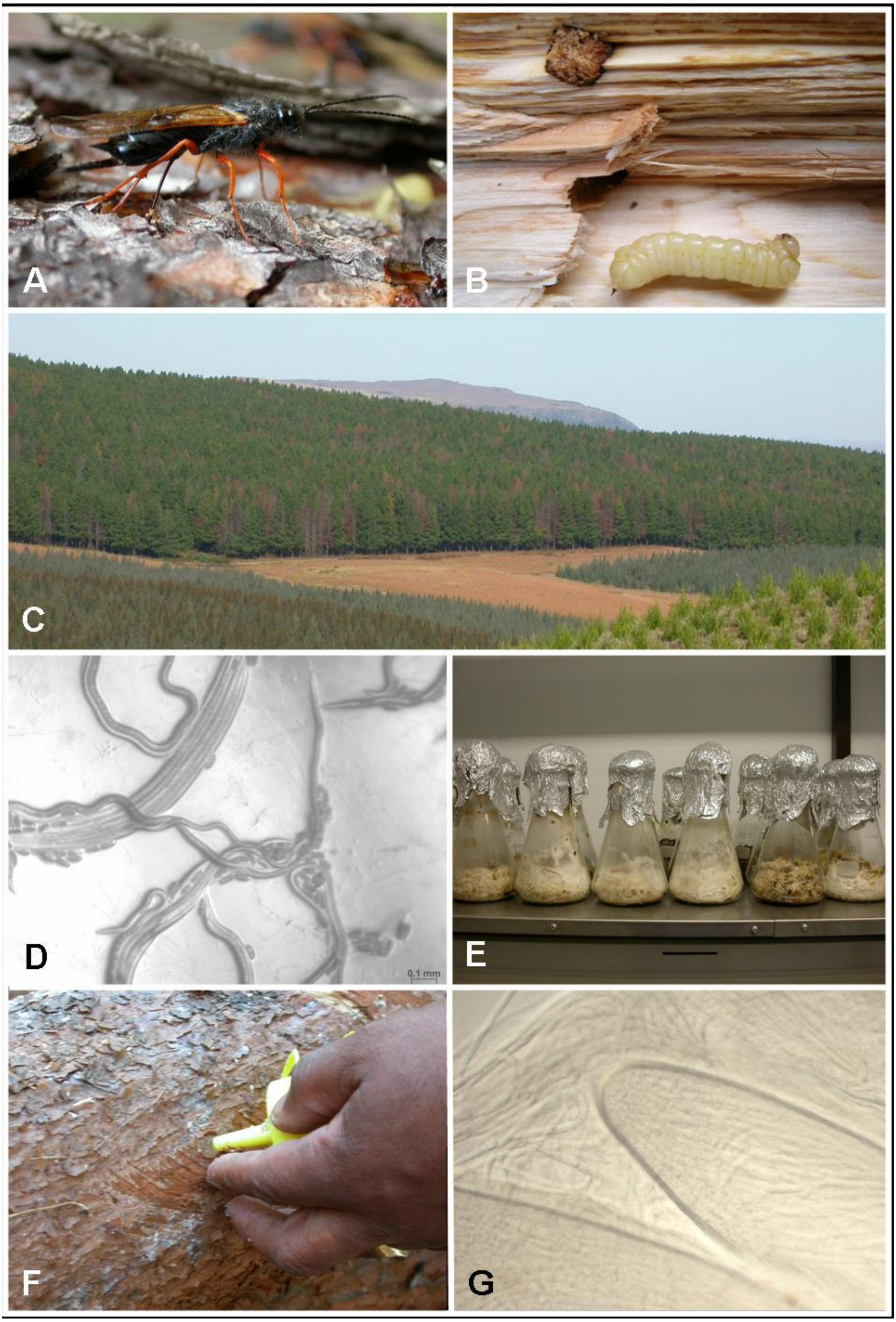
Table 2. Comparison of silvicultural practices used in *Pinus* plantations in the southern hemisphere[a,b].

	Initial stand density (spha)	Thinning	Final stand density (spha)	Pruning	Harvest age (years)	Main market for timber
KwaZulu-Natal (South Africa)	1111-1667	none	approx. 800 - 1250	for access only	15-20	pulp
Western Cape (South Africa	1111	8 years, to 650 spha (optional); 13 years, to 400 spha; 18 years, to 250 spha	250	3-4 prunes, last prune to 7.0 m	27-35	sawn timber
Chile	1100-1330	9 years, to 800 spha; 11/12 years, to 400-500 spha	400-500	2-3 prunes, last prune to 5.2 m	18-22	pulp and sawn timber
Brazil	1600	8 years, to1040 spha; 12 years, to 580 spha; 16 years, to 350 spha	350	3 prunes, last prune to 7.0m	20	pulp and sawn timber
Uruguay	1250	4 years, to 800 spha; 9 years to 450 spha	450	3 prunes, last prune to 5.5 m	20	sawn timber
Argentina	1400-1450	7 years, to 700-800 spha; 10-12 years, to 450 spha	450	varies, but often none	22	pulp and sawn timber
Australia (New South Wales)	1100	15 years, to 450 spha; 23 years, to 200-250 spha	200-250	rare, but sometimes 3 prunes at age 7, 8 and 9	32-35	sawn timber

[a] Silvicultural practices differ between land owners within the same country. While these figures therefore do not represent all the silvicultural practices in each country, it does indicate general trends.

[b] Information obtained from R. Ahumada and V. Klasmer for South America, A. Carnegie for Australia, and P. Croft, G. Boreham and D. Carstens for South Africa (pers. comm.).

Figure 1. *Sirex noctilio*, its damage and the biological control programme used in the southern hemisphere. A. Female *S. noctilio* wasp ovipositing in a *Pinus* tree. B. A typical siricid larva and larval tunnels with tightly packed frass. C. Mortality in a *Pinus patula* compartment in KwaZulu-Natal, South Africa, after heavy attack by *S. noctilio*. D. The mycetophagous stage of the parasitic nematode *D. siricidicola*, used as a biological control agent for *S. noctilio*. E. Mass rearing of *D. siricidicola* for release in the field. F. The nematodes suspended in a gel mixture, inoculated into Sirex-infested trees. G. The parasitic stage of *D. siricidicola* inside the eggs of *S. noctilio*.


A
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0.1 mm
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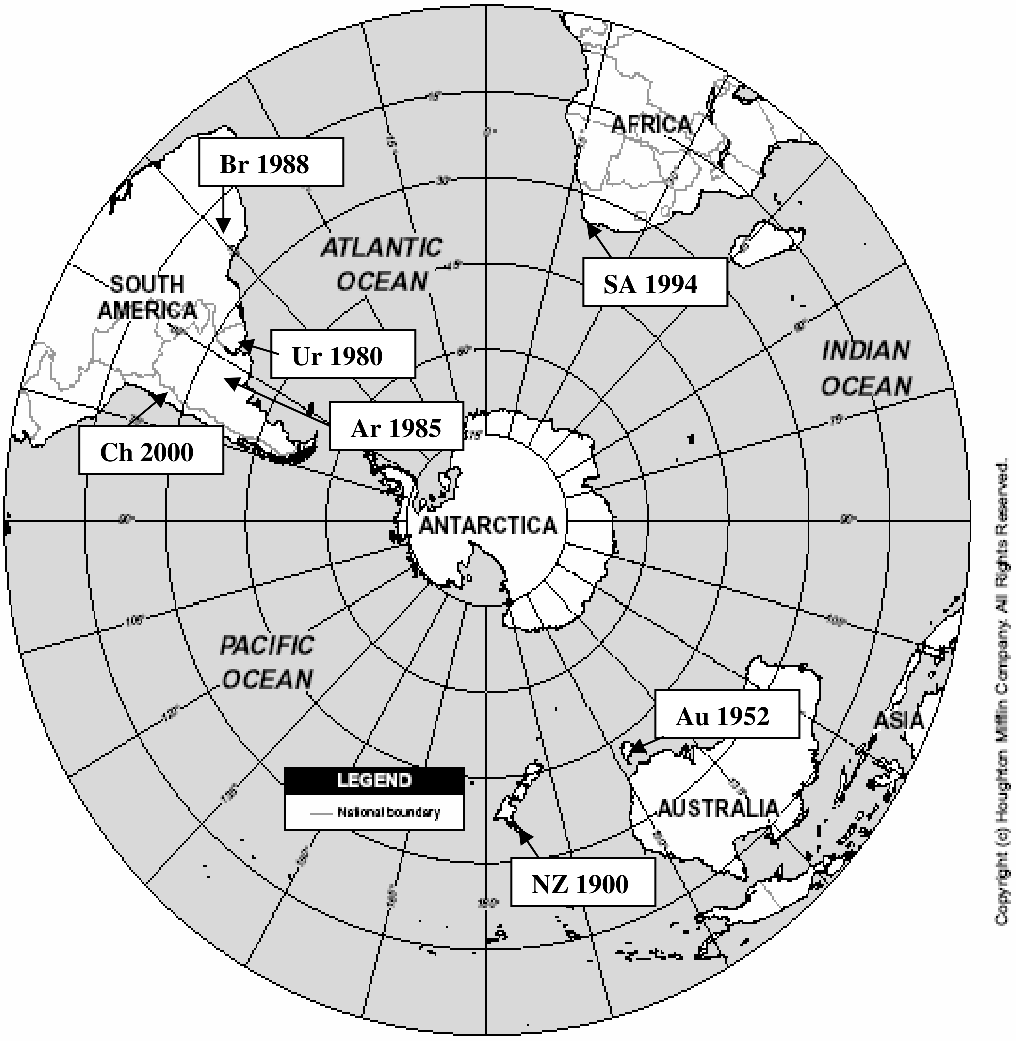
Figure 2. Detection of *S. noctilio* in the southern hemisphere. Countries are indicated by the following letters: Ar = Argentina; Au = Australia; Br = Brazil; Ch = Chile; NZ = New Zealand; SA = South Africa; Ur = Uruguay. The number following the letters indicates the date *S. noctilio* was first detected in those countries. (Map obtained from http://www.eduplace.com/ss/maps/pdf/s_hemis.pdf. Copyright © Houghton Mifflin Company. Reprinted by permission of Houghton Mifflin Company. All rights reserved. Any further duplication is strictly prohibited unless written permission is obtained from Houghton Mifflin Company.)

Southern Hemisphere



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CHAPTER TWO

Factors influencing parasitism of *Sirex noctilio* (Hymenoptera: Siricidae) by the nematode *Deladenus siricidicola* (Nematoda: Neotylenchidae) in summer rainfall areas of South Africa

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Control of the invasive wasp, *Sirex noctilio* Fabricius using the parasitic nematode *Deladenus siricidicola* Bedding is a well known example of a successful classical biological control program. Despite its wide-scale success, this control method has recently had poor success in the summer rainfall areas of South Africa. Data from previous studies showed variation in nematode parasitism from inoculated trees (inoculation success) between different tree sections and amongst inoculation times. They also pointed to moisture content of the wood or virulence of the nematode as the most likely underlying factors influencing variations in inoculation success. The results from our study showed that the highest levels of parasitism were obtained from early inoculations and from the bottom sections of trees, where moisture content of the wood was highest, supporting the hypothesis that moisture content influences parasitism. However, even when moisture content was adequate, average inoculation success remained below 25 % and was often 0 %, suggesting that there are other barriers to inoculation success. Different sources from which the nematodes were produced did not influence inoculation success, indicating that nematode virulence is most likely not the cause of the low success. Another interesting finding was that parasitized wasps were larger than unparasitized wasps. Background parasitism was present despite the poor success with past inoculations, but the data also suggest that the natural build-up of this population could be constrained by the same factors that influence inoculations.

Introduction

The woodwasp, *Sirex noctilio* Fabricius (Hymenoptera, Siricidae), is native to Eurasia (Spradbery and Kirk 1978), but during the course of the last century, it has been accidentally introduced into various southern hemisphere countries. These include New Zealand (about 1900), Australia (1952), Uruguay (1980), Argentina (1985), Brazil (1988), South Africa (1994) and Chile (2000) (Miller and Clarke 1935, Gilbert and Miller 1952, Tribe 1995, Iede *et al.* 1998, Klasmer *et al.* 1998, Maderni 1998, Ahumada 2002, Hurley *et al.* 2007a). Most recently, in 2005, an established population of *S. noctilio* was confirmed in the United States of America and Canada (Hoebeke *et al.* 2005, de Groot 2007). In these countries, *S. noctilio* has become a pest in commercial plantations and native forests, where, together with its fungal symbiont *Amylostereum areolatum* (Chaillet) Boiden, it infests and kills *Pinus* spp. (Talbot 1977).

Biological control is the strategy most commonly used to manage *S. noctilio* in pine plantations of the southern hemisphere. In particular, the parasitic nematode *Deladenus* (=*Beddingia*) *siricidicola* Bedding is considered the primary biological control agent for the pest (Bedding and Iede 2005). *Deladenus siricidicola* was first discovered in 1962 parasitizing *S. noctilio* on the North Island of New Zealand, where it was unintentionally introduced together with *S. noctilio* from Eurasia (Zondag 1969). During the course of the next decade, considerable efforts were made to screen for species and strains of *Deladenus* that resulted in high levels of parasitism and to develop effective methods to deliver them to trees (Zondag 1971, Bedding and Akhurst 1974, 1978, Zondag 1979; Bedding and Iede, 2005). This research resulted in the selection of a strain of *D. siricidicola* from Sopron, Hungary, referred to as the Sopron strain. Trees inoculated with this strain in Australia achieved parasitism levels of almost 100 % (Bedding and Akhurst 1974).

A loss of virulence in laboratory cultures of the Sopron strain, detected during the Green Triangle outbreak in south-eastern Australia (1987-1990), led to the collection and establishment of a new culture of *D. siricidicola* for laboratory breeding and release (Haugen and Underdown, 1993). These nematodes were collected from the Kamona forest in Tasmania, where the Sopron strain had been previously released. With the exception of New Zealand, this Kamona strain of *D. siricidicola* has been used throughout the southern hemisphere where *S. noctilio* has been introduced (Hurley *et al.* 2007a).

Although *D. siricidicola* has become well established in Australia, its success has been variable in South America and South Africa (Hurley *et al.* 2007a). In the summer rainfall area of South Africa in particular, nematode parasitism in inoculated trees (inoculation success) with the Kamona strain has been very poor. The first 2 years of inoculation in the province of KwaZulu-Natal in 2004 and 2005 resulted in parasitism below 5 % and 10 %, respectively. This was despite considerable efforts to streamline rearing, transport and inoculation methods for the 2005 inoculations. These disappointing results suggested strongly that the inoculation technique was not the main cause for the low levels of parasitism (Hurley *et al.* 2007a).

The reasons for the low level of success with *D. siricidicola* in South Africa are unknown. The 2004 and 2005 inoculations in KwaZulu-Natal showed a possible influence of the part of the tree inoculated and the time of inoculation on parasitism (Hurley *et al.* 2007b). This could potentially be related to differences in moisture content of the wood over time and within trees. For example, results from 2005 inoculations indicated that parasitism obtained from the bottom section of trees was higher than that from the middle and top sections. These results suggest that it would be more cost effective to inoculate the bottom section of standing trees, rather than the conventional method of felling trees to inoculate the entire boles of trees, as described in Bedding and Iede (2005).

Another factor that could have resulted in low levels of parasitism in South Africa could be a low level of viability of the nematodes used for the KwaZulu-Natal inoculations. For these inoculations, the nematodes were imported from Australia and reared in South Africa for 3 and 15 months for the 2004 and 2005 inoculations, respectively. *Deladenus siricidicola* has been known to lose its ability to convert to the parasitic form when reared in the laboratory for long periods (Haugen and Underdown 1993, Bedding and Iede 2005), and such a loss of conversion to this form could have occurred in South Africa.

Sirex noctilio is currently the most important pest of *Pinus* spp. in South Africa that seriously threatens the forestry industry. Losses due to *S. noctilio* in the summer rainfall area of South Africa have been estimated to be approximately ZAR300 million (approximately US $45 million) per year (Hurley *et al.* 2007a) and it is, therefore, crucial to achieve an effective control strategy for *S. noctilio*. The causes of the low inoculation success in KwaZulu-Natal need to be understood to determine whether these obstacles can be overcome, and thus whether the Kamona strain of *D. siricidicola* can be an effective biological control agent for *S. noctilio* in the area. The aim of our study was to build on preliminary data to better understand the influence of tree section, inoculation time, nematode source (potential influence of virulence) and moisture content on inoculation success in the summer rainfall region of South Africa. The feasibility of inoculating standing trees in this region was also considered. Furthermore, the influence of these factors on the size and numbers of emerging *S. noctilio* wasps, and how this could influence parasitism success, was investigated.

Materials and Methods

Sites

The influence of site on parasitism was not examined in this study. However, the experiment was established at two sites as a precaution against one site being lost to fire or other causes. Both sites were in the KwaZulu-Natal province, South Africa. Site 1 was a 60.2 ha *Pinus patula* Scheide et Deppe compartment, planted in January 1991, located near Underberg (29˚53′25″S 29˚23′50″E). The site was inoculated with *D. siricidicola* in 2004 (25 trees inoculated) and 2005 (88 trees inoculated). Inoculation success was 0 % in 2004 and 6.4 % in 2005. Site 2 was a 74.5 ha *P. patula* compartment, planted in June 1989, located near Boston (29˚40′16″S 29˚58′12″E). There have been no previous inoculations with *D. siricidicola* at this site.

Inoculation with *D. siricidicola*

Trees were inoculated during three periods. These were from 28 February to 1 March 2006 (Period 1), from 11 to 12 April 2006 (Period 2), and from 30 to 31 May 2006 (Period 3). Four nematode sources were used and these were all of the Kamona strain. The Australian source was obtained directly from the company that supplies the nematode used successfully for inoculations in Australia (thus considered as a control for high virulence). The FABI source was from the rearing cultures of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. These were also the nematodes used for the 2004 and 2005 inoculations in KwaZulu-Natal. The KZN source was nematodes isolated from parasitized wasps that emerged from plantations in KwaZulu-Natal, South Africa, from

October 2005 to January 2006. The Cape source was of nematodes isolated from parasitized wasps that emerged from plantations in the Western Cape, South Africa, from November 2005 to March 2006. The control trees were considered as the fifth ‘source’. These trees were not inoculated with any nematodes. All nematode sources were used at each inoculation period, except the Cape source that was not used for inoculation Period 1, because sufficient numbers of this source of nematodes were not available at that time.

Twelve trees were used for every combination of site, inoculation period and nematode source (Table 1). The trees were inoculated following the standard procedures described by Bedding and Iede (2005). Trees are felled and de-branched and a specifically designed hammer is used to make inoculation holes of approximately 10 mm deep and 30 cm apart. These holes are made in 2 rows down the length of the tree where the tree diameter is greater than 15 cm and 1 row where the diameter is less than 15 cm. Nematodes suspended in a polyacrylamide gel are squeezed into each inoculation hole, with approximately 2000 nematodes per hole. The control trees were prepared in the same manner to all other trees, but were not inoculated with nematodes. At Site 1, an additional 12 trees per combination of site, inoculation period and nematode source were used. These trees were not felled prior to inoculation and only a 1.5 m section of the tree, from breast height (approximately 1.5 m from ground level) upwards, was inoculated. This treatment was included to compare parasitism in felled versus standing trees. Thus, a total of 336 felled trees and 168 standing trees were inoculated (Table 1).

Moisture measurements of trees

The moisture content of the inoculated and control trees was measured with a Bes Bollmann moisture probe (model H D1 3.10, Gottmadingen, Germany). The probe was inserted so that the tips penetrated approximately 45 mm into the wood. The probe tips were 8 mm long, and thus the measurements were taken at a depth between 442 mm and 450 mm. These measurements were taken over the bark. For each measurement, the probe was inserted three times within a 10 cm area and the mean of the three moisture values was considered as the moisture reading for that point. For the felled trees, moisture measurements were taken from the mid-point of the bottom, middle and top section of each tree. The bottom, middle and top sections of the trees were defined as the first, second and last third of the tree, respectively. This was after the tree had been felled and the top excised where the stem diameter was 5 cm, as described by Bedding and Iede (2005). For the standing trees, moisture measurements were taken only from the bottom section of the stems. These measurements were taken at breast height, approximately 1.5 m above ground level.

Moisture measurements were taken from the time the trees were inoculated until just prior to the samples being collected. For trees inoculated in the first inoculation period, moisture measurements were taken after 0, 41, 86, 125, 161 and 185 days (28.02.2006, 10.04.2006, 25.05.2006, 03.07.2006, 08.08.2006 and 01.09.2006, respectively), and these time points were referred to as MTIME 1 to 6, respectively. Trees that were inoculated in the second inoculation period did not have moisture measurements taken for MTIME 1, and trees inoculated in the third inoculation period did not have moisture measurements taken for MTIME 1 and 2.

To ensure that measurements taken with the moisture probe were accurate and meaningful, they were compared to the conventional oven-dry method of measuring moisture content. Thus, 62 discs of approximately 10 cm thickness were collected from trees at Site 2 on 5 September 2006. Directly after taking moisture readings from these discs with the moisture probe, the discs were weighed, placed in an oven overnight, and weighed again. The oven-dry measurements were calculated as percentage moisture content equal to oven dry weight over fresh weight x 100.

Collection of billets

Samples from the inoculated trees were collected on 5 September 2006. One 80 cm to 90 cm billet was collected from the bottom, middle and top sections of every inoculated and control tree. These billets were placed in emergence drums. The emergence drums were 210 L metal drums with one end open. Netting in the shape of a wind sock with an opening that was closed with an elastic band was placed over the open end of the drum. Wasps would move towards the netted side of the cage, as they were attracted to the light, and were then collected via the opening in the net. Three billets were placed in each emergence drum. Billets with the same combination of site, inoculation period, nematode source and tree section, and with similar moisture content, were placed in the same emergence drums. Thus, a total of 1512 billets were collected and stored in 504 emergence drums (Table 1). The emergence drums were stored in a shaded facility in KwaZulu-Natal.

Dissection and measurement of emerging *S. noctilio* wasps

Wasps emerging from the drums were collected and transported to the laboratories of FABI, Pretoria, where they were dissected. Only female *S. noctilio* wasps are involved in moving *D. siricidicola* to freshly attacked trees, thus ensuring the survival of the nematodes. Consequently, female parasitism is viewed as the most accurate indication of the nematodes' influence on *S. noctilio* populations. Dissections, therefore, focused on female wasps, although a proportion of the male wasps were also dissected to ensure sufficient data for the analyses. Wasps were dissected and the eggs of female wasps and the testes of male wasps examined for the presence of *D. siricidicola*, using light microscopy. The lengths of 2611 emerging wasps were measured from the tip of the head to the tip of the cornus (short dorsal spine at the apex of the abdomen).

Statistical analysis

The number of wasps emerged, dissected and parasitized, per drum per day, was used for the statistical analyses of the emergence and parasitism of *S. noctilio*. For the moisture data, the mean of the three measurements taken per section for each tree were used. An Analysis of Variance (ANOVA) was performed on the data using a General Linear Model (GLM). To address the questions at hand, more than one GLM model was used. This was because the data for some of the class variables were not evenly distributed. For example, the Cape nematode source was not used for the first inoculation period, but the other nematode sources were used; only trees inoculated in the first inoculation period had moisture measurements taken for MTIME 1 and MTIME 2; and no moisture measurements were taken for the middle and top of standing trees until MTIME 6. A Bonferroni Correction was applied to the p-values to reduce Type I errors in multiple-hypothesis testing. An ANOVA using a GLM procedure was also used to analyze the wasp size data. Means used from the GLM analyses are given as Least Squares Means (LS means), as LS means better reflect the model used for the data than unchanged means.

Correlation and regression analyses were performed to examine the relationship between parasitism and moisture content. For these analyses the mean parasitism values over time per drum and the corresponding mean moisture content values over time were used. Correlation and regression analyses were also performed to examine the relationship between moisture content values obtained with the moisture probe and with the oven-dry method. SAS version 8.2 (SAS Institute, 2001) was used for all statistical analyses.

Results

General

In total 49849 wasps (45983 males, 3866 females) emerged from the collected billets. Of these 17823 wasps (14062 males, 3761 females) were dissected. The average parasitism obtained for the experiment from male and female wasps was 3.7 % (5.8 % female parasitism), including the control billets, or 4.3 % (7.2 % female parasitism) excluding the control billets. Female parasitism was higher than male parasitism, with an average of 7.2 % female parasitism obtained in the experiment, excluding control billets, compared to 3.5 % male parasitism. The sex-ratio was strongly biased towards the males, with approximately 1: 12 females to males.

Wasp emergences from drums commenced on 20 October 2006 and ended on 18 January 2007. A small number of wasps emerged after this period and they were not included in the analyses. Male and female wasp emergences peaked from 10-23 November 2006. There was a general trend for parasitism to increase over emergence time, for both male and female parasitism

Correlation between moisture probe and oven-dry method

A Pearson’s Correlation was used to test for the association between moisture content values obtained with the moisture probe and values obtained using the oven-dry method, regarded as actual moisture content values. The association between the two methods was high (correlation coefficient = 0.89, $p < 0.0001$), providing confidence in the values obtained using the moisture probe. Furthermore, a regression analysis was done to determine the nature of this relationship (r-square = 0.79):

$$y = 5.72 + b(0.72)$$

where y is the actual moisture content value (obtained if the oven-dry method was used), and b the value obtained using the moisture probe.

Moisture content over time

For all tree sections, moisture content decreased from the time of inoculation to the time when the billets were collected (Fig. 1). Both sites were in a summer rainfall area, with May to August being dry months. Thus the decrease in moisture content was likely related to the decrease in rainfall. Moisture content was lowest at MTIME 5 (8 August), where mean moisture content was 47.3 % for the bottom section, 19.6 % for the middle section and 15.3 % for the top section (Fig. 1). Moisture increased between MTIME 5 and MTIME 6 (1 September), possibly due to the onset of the rainy season between these periods (Fig. 1).

Site

Parasitism obtained from trees that were felled and inoculated was below 10 % for both sites (Table 2). There was no significant difference for total parasitism (from male and female wasps) between the sites ($F = 7.85$; $df = 2, 3660$; $P = 0.25$). There was a significant difference in emergence numbers between the two sites, with Site 2 having significantly more males (*F* = 28.12; *df* = 2, 31584; *P* = 0.002), females (*F* = 28.12; *df* = 2, 31584; *P* < 0.001) and total wasps (*F* = 28.12; *df* = 2, 31584; *P* < 0.001) emerging on average than Site 1 (felled trees only). The sex-ratio was 1:10 females to males at Site 2 and 1:13 at Site 1. The average moisture content over time, from felled trees, was significantly higher at Site 1 (LS mean = 39.6 %) than at Site 2 (LS mean = 36 %) (*F* = 77.59; *df* = 1, 4200; *P* < 0.0001).

Nematode source and control

The different nematode sources had no significant effect on total parasitism (*F* = 0.91; *df* = 3, 3476; *P* = 0.43). Total parasitism of 1.7 % was obtained from the control trees (Table 2), which were not inoculated. These nematodes would have been naturally placed in the tree by *S. noctilio* wasps infected from the previous 2 years' inoculations in the surrounding area. Parasitism from control trees was lower than that obtained in inoculated trees, but there was not a great difference between these values. Total wasp emergences from billets inoculated with the Cape source were lower than the other sources (Table 2), as there were no trees inoculated with this source during the first inoculation (Table 1). There was no significant difference in moisture content of trees inoculated with different nematode sources (*F* = 0.72; *df* = 3, 4200; *P* = 0.54).

Inoculation time

Parasitism from trees inoculated in the first inoculation period (beginning March) was higher than that from the later two inoculation periods (Table 2). This difference in parasitism was significant when comparing total parasitism (*F* = 23.95; *df* = 2, 3660; *P* < 0.001), and excluding the Cape nematode source, which was not used for the first inoculation period and that was the source where the lowest parasitism was obtained. The differences were mainly due to differences in the middle section of the tree between the inoculation periods (Fig. 2). Parasitism levels in control trees was also higher from trees felled, but not inoculated, in the first inoculation period, compared to trees felled, but not inoculated, in the second and third inoculation periods (3.8 % compared to 0.7 % and 0.6 %), but this interaction was not significant ($F = 0.73$; $df = 6, 4956$; $P = 0.63$).

The total number of wasps emerging from billets inoculated during the first inoculation period was substantially lower than from billets inoculated during the subsequent two inoculation periods (Table 2). This was because the Cape nematode source was not used in the first inoculation period.

Moisture content in the bottom, middle and top section of the tree was significantly higher at the time of the first and second inoculation, than at the time of the third inoculation ($F = 10.53$; $df = 10, 4200$; $P < 0.0001$) (Fig. 1). Moisture content values taken from MTIME 3 onwards, when trees from all inoculation periods had been inoculated, were significantly higher for trees inoculated in the first inoculation period than for trees inoculated in the later inoculation periods. This was for both the middle and top tree sections, but not for the bottom section ($F = 14.31$; $df = 2, 1073$; $P < 0.001$).

Tree section

For both felled and standing trees, parasitism was highest in the bottom section of the tree and lowest in the top section (Table 2). However, the ranking of parasitism between tree sections of felled trees differed between the two sites (Fig. 3). At Site 2, the highest parasitism was obtained from the bottom section, while at Site 1 the highest parasitism was obtained from the middle section. For both sites, the lowest parasitism was obtained from the top section, and although this difference was not significant at Site 1 considering the Bonferroni correction, it was still considerable. As expected, parasitism from the middle and top sections of standing trees was very low, as only the bottom section was inoculated. The same ranking of parasitism between tree sections for the different sites was observed from control trees.

Most wasps emerged from the middle sections of the trees for both felled and standing trees (Table 2). These differences were significant when comparing total wasp emergences over both sites ($F = 316.01$; $df = 2, 31584$; $P < 0.01$). However, significantly more female wasps emerged from the bottom sections than the middle or top sections ($F = 103.73$; $df = 2, 31548$; $P < 0.0001$) (Table 2).

Moisture content was significantly higher in the bottom sections of the trees than the middle sections and significantly higher in the middle sections than the top sections ($F = 1650.56$; $df = 2, 4200$; $P < 0.0001$). This pattern was consistent over time (Fig. 1). When comparing the moisture content of felled trees between the two sites, moisture content in the middle and top sections was significantly higher at Site 1 than at Site 2 (Fig. 4).

Influence of moisture content on parasitism

The numerous zero and low parasitism values obtained across the range of moisture content values showed that the relationship between moisture content and parasitism was weak (see Fig. 5 for example). The Pearson's Correlation Coefficient between moisture content and total parasitism was below 0.1 for the bottom, middle and top section. The relationship between moisture content and parasitism for specific inoculation periods was also examined, but again the relationship between these variables was poor, with the strongest relationship being from the middle tree section during the first inoculation period (Pearson's

Correlation Coefficient = 0.37, p = 0.039) and the bottom tree section during the third inoculation period (Pearson's Correlation Coefficient = 0.36, p = 0.005) (Table 3).

Size of emerging *S. noctilio*

The lengths of the 2611 wasps that were measured ranged from 10-44 mm. Overall, the average parasitized wasp (32 mm) was significantly larger than the average wasp that was not parasitized (24 mm) (*F* = 15.28; *df* = 1, 2601; *P* < 0.0001). There was a significant difference in the size of wasps that emerged from different tree sections. Wasps that emerged from the bottom sections were significantly larger than wasps that emerged from the middle sections, and wasps that emerged from the middle sections were significantly larger than wasps that emerged from the top sections (*F* = 10.04; *df* = 2, 2601; *P* < 0.0001). When comparing the size of parasitized and non-parasitized wasps within each tree section, parasitized wasps were larger than wasps not parasitized for the bottom (parasitized = 35 mm (n = 112), unparasitized = 28 mm (n = 1228)), middle (parasitized = 27 mm (n = 30), unparasitized = 24 mm (n = 1172)) and top (parasitized = 33 mm (n = 3), unparasitized = 22 mm (n = 646)) tree sections, although the interaction between tree section and parasitism was found to be non-significant (*F* = 1.28; *df* = 2, 2601; *P* = 0.2770).

Felled versus standing trees

Parasitism was higher in trees that were felled and inoculated as opposed to trees that were inoculated standing (Table 2). This was as expected, as only the bottom section of standing trees had been inoculated. When only the bottom sections of the trees were compared, parasitism remained higher in felled trees (Table 2), but this difference was not significant when only comparing felled trees at Site 1 with standing trees at the same site (*F* = 8.16; *df* = 4, 3660; *P* = 0.97). A low level of parasitism (less than 1 %) was found in the middle sections of standing trees (Table 2), although this section had not been inoculated. This parasitism could have resulted from the upward movement of nematodes inoculated in the bottom sections, or from nematodes naturally placed in the tree by *S. noctilio* females, i.e. background parasitism. Because total parasitism from the middle sections of standing trees that had not been inoculated (control trees) was 1.1 %, it is likely that at least the majority of the parasitism from the middle section of inoculated trees resulted from background parasitism. No parasitism was found in wasps from the top sections of standing trees.

More wasps emerged from standing trees than from felled trees (Table 2), when comparing the same site, i.e. Site 1. This difference was significant for the male (*F* = 27.2; *df* = 2, 31584; *P* < 0.001) and total emergences (*F* = 28.12; *df* = 2, 31584; *P* < 0.001). The sex-ratio for the standing trees at Site 1 was 1:14 females to males, compared to 1:13 for the felled trees at the same site.

The average moisture content in the bottom section of standing trees was significantly lower than that for felled trees at the same site (*F* = 71.89; *df* = 2, 1619; *P* < 0.001). This difference was due to the difference in moisture content for readings taken at MTIME 4 to 6, where moisture content was 37 %, 44 % and 34 % for standing trees, and 46 %, 51 % and 48 % for felled trees, respectively. Moisture readings taken at MTIME 6 for the middle and top tree section, were also significantly lower for standing trees (middle = 17.7 %, top = 13.1 %) than for felled trees (middle = 28.2 %, top = 20.2 %) (*F* = 71.18 (middle), 81.66 (top); *df* = 2, 395; *P* < 0.001).

Discussion

Attempts at biological control of *S. noctilio* using the parasitic nematode *D. siricidicola* in summer rainfall areas of South Africa have been largely unsuccessful. Hurley *et al.* (2007a) suggested possible reasons for this lack of success. These included loss of virulence of the nematode source used and low moisture content of the wood during and after the inoculation process. Results of our study have shown that nematode virulence was not a factor in the poor success of inoculations, but that wood moisture content could substantially influence the efficacy of control using this nematode. However, the results also indicated that moisture content alone is unlikely to define nematode success and that other currently unknown factors are most probably also involved.

The emergence peak of *S. noctilio* between 10 and 23 November is similar to the emergence peak of the previous year (unpublished data). This differs from emergences recorded from Southern Argentina and Australia, where emergences were from December to May and peaked in February or March, and from the Western Cape, where emergences were from mid-November to May and peaked in March (Haugen *et al.* 1990, Klasmer *et al.* 1998, Tribe and Cillié 2004). Differences in emergence period most likely reflect differences in climate between various areas.

Parasitism rates from inoculated trees in this study were very low, and similar to those obtained from previous year's inoculations. The source from which the nematodes were produced did not have a significant effect on parasitism. This included the use of the same Australian nematode source that recently attained total parasitism rates of over 75 % in New South Wales, Australia (Carnegie *et al.* 2005). The low parasitism rates obtained from all nematode sources in this experiment, including the Australian nematode source, indicates that the virulence of the nematodes used in South Africa from 2004 to 2006 is unlikely to be the cause of the low parasitism obtained in the summer rainfall areas of the country.

The time of year that trees were inoculated and the tree section inoculated had a significant effect on parasitism. The highest parasitism rates were obtained from trees inoculated at the beginning of March and in the bottom and middle sections (for felled trees). Moisture content was highest in the bottom sections of the trees and from the first inoculation, where parasitism was highest. Furthermore, moisture content and parasitism from the middle tree sections were significantly higher from Site 1 than from Site 2. These findings suggest that moisture content has a substantial effect on parasitism.

The means by which moisture content of the wood might influence parasitism is not known. Moisture content could directly affect the survival and mobility of *D. siricidicola*. Alternatively, moisture content could affect the establishment of the symbiotic fungus *A. areolatum* on which *D. siricidicola* feeds. This would be consistent with the view of Taylor (1981) who noted that *A. areolatum* growth is impeded by an excess or scarcity of water.

Knowledge of the influence of moisture content on *D. siricidicola* and *A. areolatum* is not new. For example, Zondag (1969) recognized that more nematodes were present in moist wood when *D. siricidicola* was first used as a biological control agent. Likewise, Bedding and Akhurst (1974) noted that *D. siricidicola* requires wood moisture content of 50 % and higher for successful establishment. In contrast, Haugen and Underdown (1993) concluded that moisture content was not a major factor causing low levels of parasitism in *P. radiata* billets inoculated in Australia. Moisture content of these billets ranged from 33 % to 72 %, with a mean of about 45 %. However, for the majority of the period from when the trees are inoculated with the nematodes to when the wasps emerged from the trees, the moisture content in the middle and top sections of trees inoculated in KwaZulu-Natal are well below values suggested by Bedding and Akhurst (1974) and tested by Haugen and Underdown

(1993) (Fig. 1). Such low moisture content could negatively influence the survival and movement of *D. siricidicola* and/or the establishment of *A. areolatum*.

Although low moisture content appears to be a barrier to parasitism, it is not the only barrier to success of parasitism in the summer rainfall region of South Africa. This is evident from the low statistical correlation between these variables and the poor predictability of their relationship. Although most parasitism was obtained where moisture content was higher, high moisture content did not guarantee high parasitism levels. Inoculations in the first inoculation period and in the bottom tree sections, where moisture content should not have been a limiting factor, still only gave female parasitism rates less than 25 % (Table 4), with many bottom sections obtaining 0 % parasitism (Fig. 5). It is not clear which other factors are responsible for the low parasitism rates. Some possibilities include the presence of bluestain fungi in the trees which may compete with the fungal food source of the nematodes, namely *A. areolatum*, or incompatibility between nematode and fungus or nematode and wasp strains (King 1966, Hurley *et al.* 2007a).

The reasons for higher moisture content in felled trees as compared to standing trees, as well as higher moisture content in trees felled earlier as compared to trees felled later are not known. One possible explanation could be that standing trees lose moisture through their needles, which does not occur in felled trees which are de-branched. Furthermore, felled trees would be less exposed to sunlight than standing trees.

Focusing inoculations where moisture content is adequate should increase inoculation success substantially in the summer rainfall regions of South Africa. Data from our study show that it is optimal to inoculate the bottom and middle sections of felled trees in the first inoculation period, with 15.1 % of the female *S. noctilio* from the entire tree being parasitized from inoculating these two sections (Table 4). If only the bottom sections are inoculated in the first inoculation period, 8.5 % of the female *S. noctilio* are parasitized. Inoculating the top sections of felled trees increased the parasitized females by only 1.7 %, and is probably not worth the time and resources required for the inoculations. Inoculating standing trees limits the inoculations to the bottom third of the tree and would thus be considerably less effective than inoculating felled trees. However, the cost and safety of the different inoculation techniques would need to be considered when deciding on the most feasible approach.

An interesting observation in our study was that parasitized and non-parasitized *S. noctilio* differed markedly in size. The reason for these differences is not known, but it could possibly be due to differences in *A. areolatum* establishment within trees. Where *A. areolatum* is well established, there would be a sufficient food resource for *S. noctilio* larvae to reach their full size (Madden 1981), but also to sustain larger nematode populations, thus increasing the probability that these larger larvae would become parasitized. The establishment of *A. areolatum* could be restricted by low moisture, as discussed earlier, and the presence of bluestain fungi as suggested by King (1966). Both these conditions are more prevalent in the upper parts of trees, potentially explaining why smaller *S. noctilio* and lower parasitism were observed in the top sections of the trees.

The low levels of inoculation success using *D. siricidicola* in the summer rainfall region of South Africa, even when wood moisture content is adequate, questions whether it will be feasible to use this nematode as a biological control agent in this region. One positive indication was, however, that background parasitism was found at both sites. This shows that despite the low parasitism levels in these areas for the 2004 and 2005 inoculations, *D. siricidicola* has become established, albeit at very low levels. Furthermore, there had been no previous inoculations at Site 2, indicating that infected *S. noctilio* moved from surrounding areas that had been inoculated, and possibly from other plantations. These results are encouraging, as they show the potential for *D. siricidicola* to naturally spread despite low inoculation success. It remains to be determined whether the rate at which this might occur, given low inoculation success, will be sufficient to reduce *S. noctilio* populations in this area to economically tolerable levels. Furthermore, the similarity in patterns of parasitism regarding time and tree section between inoculated and control trees suggest that the same barriers prevent high parasitism in both cases. The development of background parasitism at inoculated and un-inoculated sites requires further investigation to address these concerns.

The low moisture content at and after inoculation is an unavoidable condition in summer rainfall areas such as KwaZulu-Natal. This barrier to inoculation success can not be manipulated, but inoculations can focus on the optimal time and tree position, where parasitism will be least effected by moisture content. However, even incorporating these factors, artificial inoculation success is likely to remain low. The natural spread of the nematode should contribute to its establishment, but is clearly also affected by the same factors influencing artificial inoculations. Thus, although inoculations should continue in an effort to further the establishment of *D. siricidicola* in KwaZulu-Natal, urgent attention must be paid to understanding and addressing other barriers to inoculation success, with the ultimate aim of greatly increasing initial and background parasitism.

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Table 1. Experimental design. The experimental design included two sites with either felled or standing trees. The trees were inoculated from 28 February to 1 March 2006 (Period 1), from 11 to 12 April 2006 (Period 2), and from 30-31 May 2006 (Period 3). Four nematode sources were used, as well as uninoculated (control) trees, which were considered the 'fifth' source. Billets were collected from the treated trees and placed into emergence drums.

Site	Felled / Standing	Inoculation Period	No. of nematode sources used	No. of trees prepared	No. of billets collected (one per tree per section)	No. of emergence drums used
1	Felled	1	4	48	144	48
		2	5	60	180	60
		3	5	60	180	60
2	Felled	1	4	48	144	48
		2	5	60	180	60
		3	5	60	180	60
1	Standing	1	4	48	144	48
		2	5	60	180	60
		3	5	60	180	60
Totals				*504*	*1512*	*504*

Table 2. Summary of emergence and parasitism data for *Sirex noctilio*. Emergence columns are a summary of all the data, including control trees. Dissection and parasitism columns exclude the control trees, except for the last row

		Emerged			Dissected			% Parasitism		
		Male	*Female*	*Total*	*Male*	*Female*	*Total*	*Male*	*Female*	*Total*
Site (felled trees only)	1	14114	1122	15236	3306	801	4107	5.1	8.0	5.7
	2	14671	1478	16149	3317	1106	4423	3.4	9.2	4.9
Felled vs. Standing	Felled (Site 1 and 2)	28785	2600	31385	6623	1907	8530	4.3	8.7	5.3
	Standing (Site 1)	17198	1266	18464	4446	990	5436	2.4	4.2	2.7
Tree section (felled)	Bottom	9794	1073	10867	2368	752	3120	7.7	15.0	9.5
	Middle	12657	976	13633	2539	731	3270	3.3	6.4	4.0
	Top	6334	551	6885	1716	424	2140	0.9	1.4	1.0
Tree section (standing)	Bottom	5766	559	6325	1446	427	1873	6.3	8.7	6.8

	Middle	8045	491	8536	1849	371	2220	0.8	1.3	0.9
	Top	3387	216	3603	1151	192	1343	0.0	0.0	0.0
Inoculation period	1	10250	895	11145	3177	552	3729	7.8	13.6	8.6
	2	17254	1444	18698	4240	1177	5417	2.2	5.9	3.0
	3	18479	1527	20006	4106	1172	5278	2.1	5.8	2.9
Nematode sources	Australia	9781	817	10598	2984	802	3786	5.0	9.6	6.0
	KZN	8742	852	9594	2874	824	3698	3.0	8.3	4.1
	FABI	10379	741	11120	3225	712	3937	3.3	6.7	3.9
	Cape	6498	578	7076	1986	559	2545	2.4	2.7	2.5
	Control	10583	878	11461	2993	864	3857	1.9	1.2	1.7

Table 3. Correlation between moisture content and parasitism at time of inoculation for the different tree sections. Values were obtained using a Pearson's Correlation analysis. Where the correlation is significant ($p < 0.05$), the correlation coefficient is in italics

	Inoculation period		
	1	2	3
	0.16[a]	0.19	*0.36*
Bottom	0.27[b]	0.15	0.005
	48[c]	59	60
	0.37	0.25	0.18
Middle	0.039	0.11	0.26
	32	40	40
	0.17	-0.11	-0.11
Top	0.35	0.5	0.48
	31	39	40

[a] Pearson's Correlation Coefficient

[b] p-value

[c] number of observations

Table 4. A comparison of female parasitism obtained from inoculating different sections of felled trees. Female parasitism is expressed per tree section and for all tree sections combined (i.e. the entire tree). Data used is from the first inoculation period of this experiment. A. Percentage of females parasitized per tree section (number of infected females in tree section / total number of females from that tree section). B. Percentage of females from all tree sections combined that are parasitized from inoculating one tree section only (number of infected females in tree section / total number of females from all three tree sections). C. Percentage of females from all tree sections combined that are parasitized from inoculating the bottom and middle tree section only (number of infected females in bottom and middle tree section / total number of females from all three tree sections). D. Percentage of females parasitized from all sections combined (number of infected females from all three tree sections / total number of females from all three tree sections). Control trees are excluded.

	No. female emergences	No. parasitized females	% of females parasitized			
			A	B	C	D
Bottom	132	30	22.7	8.6	15.2	16.9
Middle	127	23	18.1	6.6		
Top	90	6	6.7	1.7		

Figure 1. Change of moisture content over time for felled trees at the bottom, middle and top tree section. MTIME refers to the period when moisture measurements were taken, as indicated in Materials and Methods. MTIME 1, 2 and 3 were moisture readings at the first, second and third inoculation time, respectively. MTIME 6 was the last moisture reading, prior to the billets being collected. The dotted lines indicate the 50 % and 35 % moisture content mark (see Discussion).

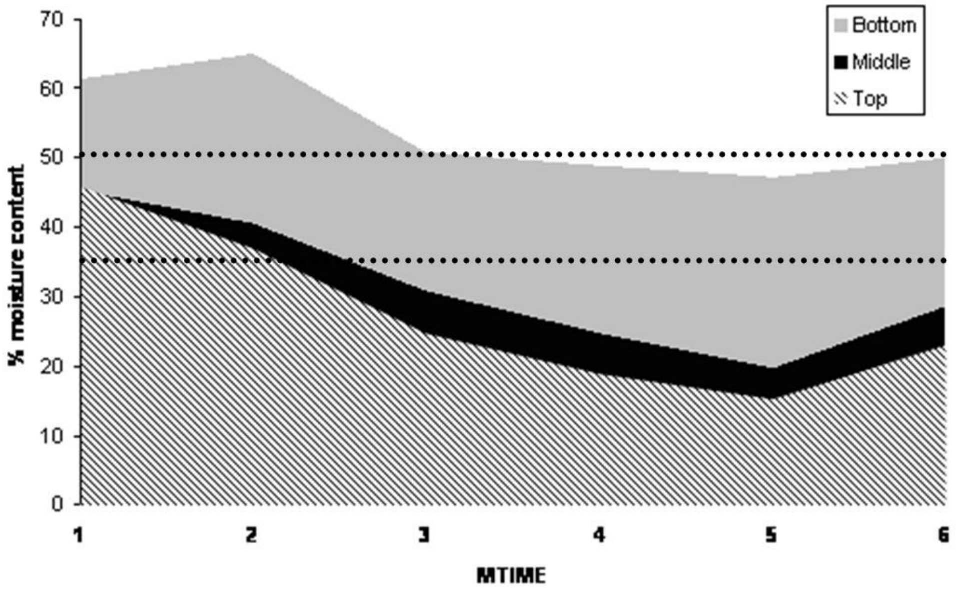

70
60
50
40
30
20
10
0
% moisture content
1
2
3
4
5
6
MTIME
Bottom
Middle
Top

Figure 2. Total parasitism for the different inoculation periods for the bottom, middle and top tree section. The same letter indicates no significant difference (Bonferroni correction = 0.0014), within each tree section. The parasitism values are Least Squares Means.

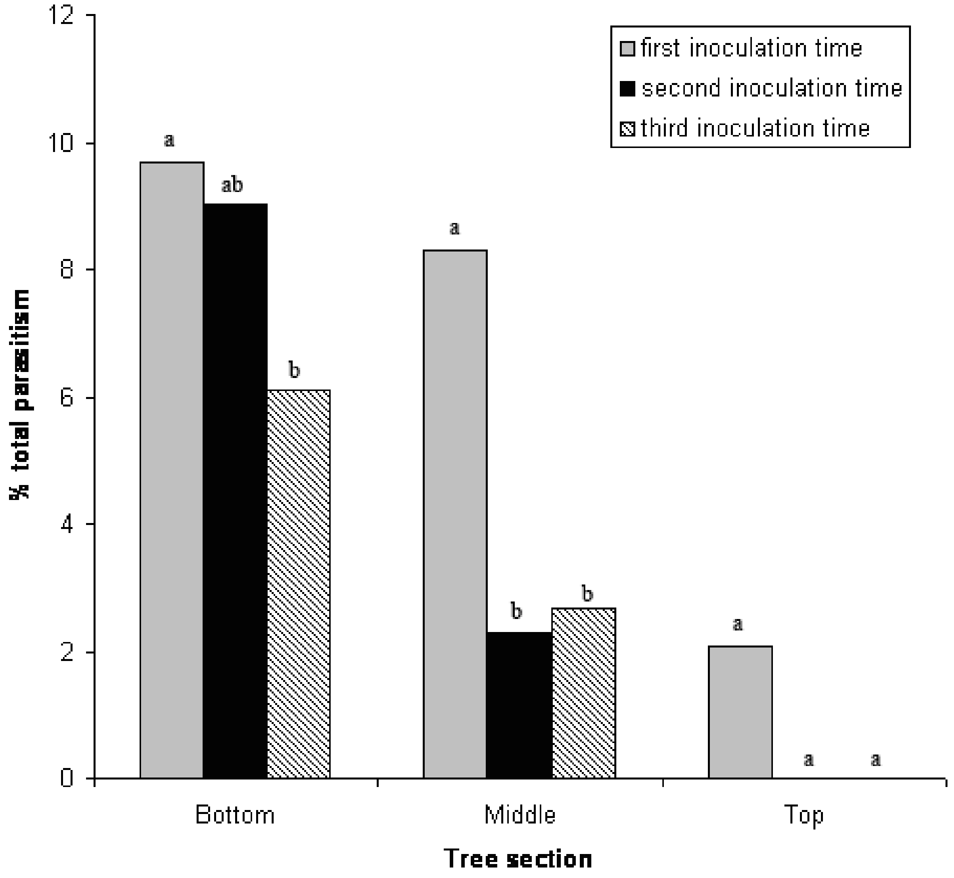

12
10
8
6
4
2
0
% total parasitism
first inoculation time
second inoculation time
third inoculation time
a
ab
b
a
b
b
a
a
a
Bottom
Middle
Top
Tree section

Figure 3. Comparison of total parasitism between tree sections at the different sites and for standing and felled trees. The same letter indicates no significant difference (Bonferroni correction = 0.0014) within Site 1 (standing), Site 1 (felled) and Site 2 (felled). The parasitism values are Least Squares Means.

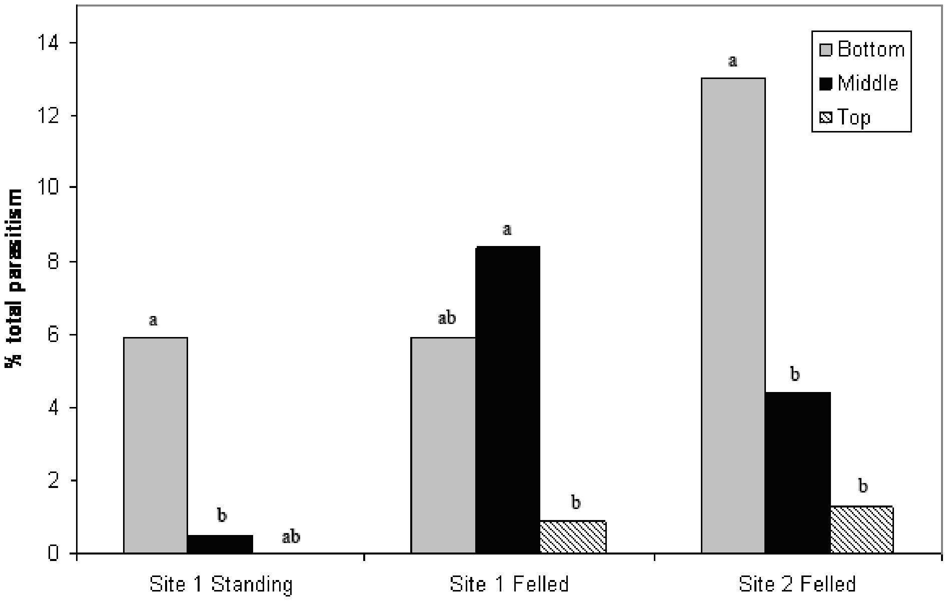

% total parasitism
0
2
4
6
8
10
12
14
Bottom
Middle
Top
a
b
ab
ab
a
b
a
b
b
Site 1 Standing
Site 1 Felled
Site 2 Felled

Figure 4. Moisture content of felled trees among tree sections for the different sites. The same letter indicates no significant difference within and between sites (Bonferroni correction = 0.003). The moisture values are Least Squares Means.

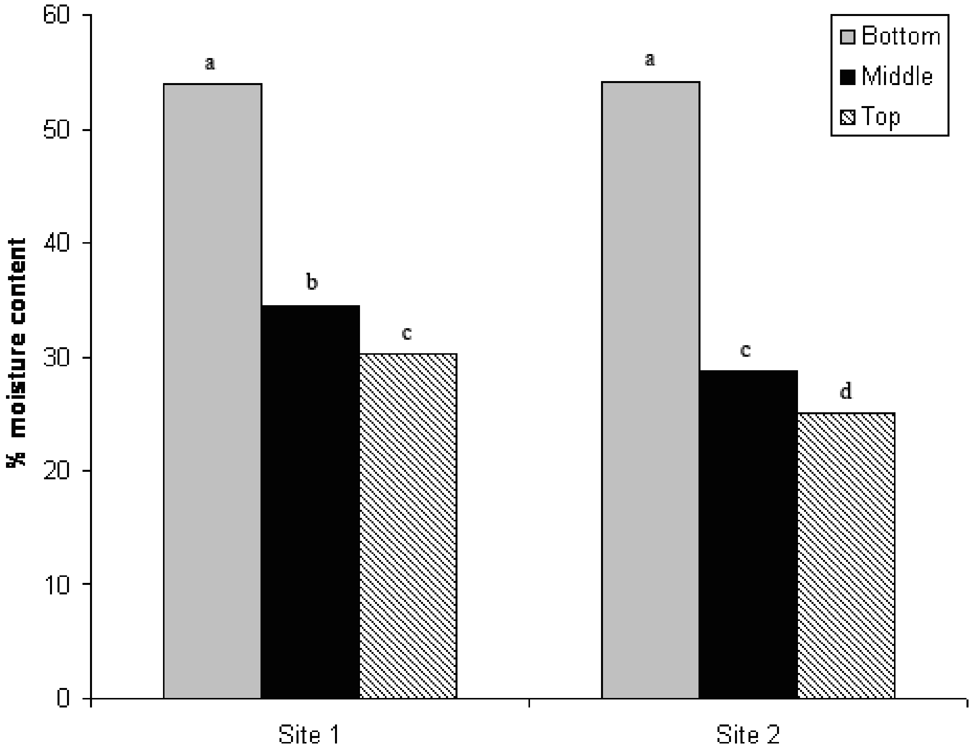
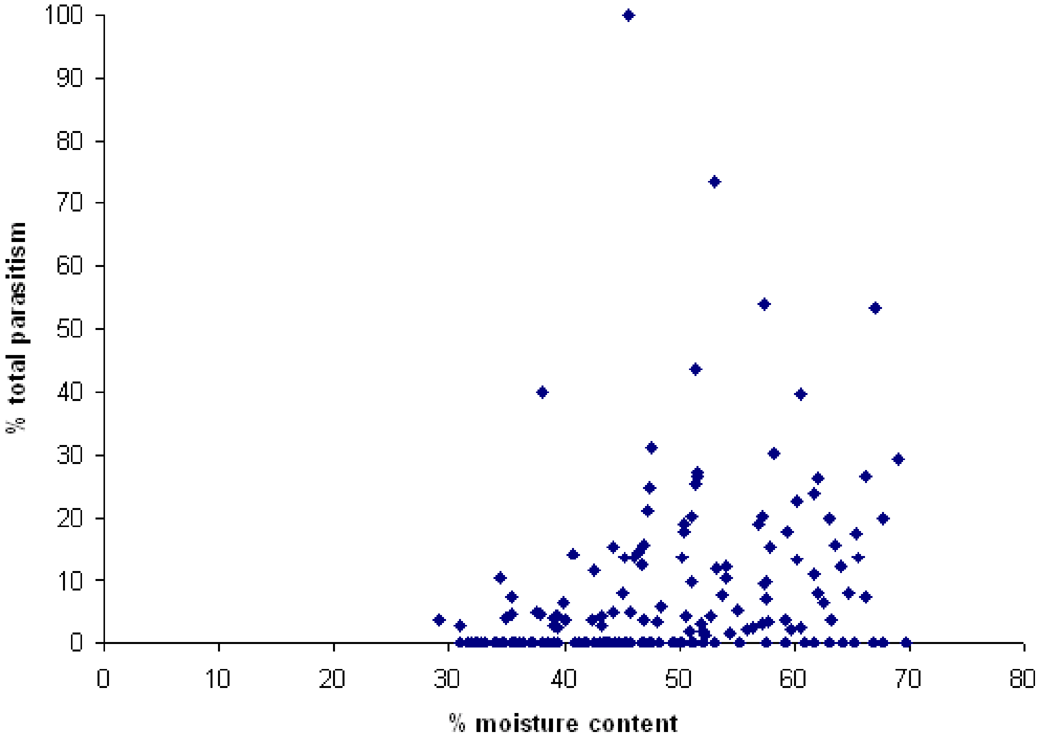

% moisture content
60
50
40
30
20
10
0
a
b
c
a
c
d
Site 1
Site 2
Bottom
Middle
Top

Figure 5. Scatter plot showing distribution of total parasitism values over moisture content, in the bottom tree section. Data were from felled and standing trees.


100
90
80
70
60
50
40
30
20
10
0
% total parasitism
0
10
20
30
40
50
60
70
80
% moisture content

CHAPTER THREE

The influence of *Amylostereum areolatum* diversity and competition on the fitness of the Sirex parasitic nematode *Deladenus siricidicola*

The *Sirex noctilio* (woodwasp) - *Amylostereum areolatum* (fungus) complex has caused substantial losses to pine industries in its introduced range. The nematode *Deladenus siricidicola* that parasitizes *S. noctilio* and feeds on *A. areolatum* is widely used as a biological control agent for *S. noctilio*, but not with consistent success. This variable success could be due to factors that influence the feeding and reproductive ability of the nematode on *A. areolatum*. In this study, we test two main hypotheses that emerge from this prediction. Firstly, we compared the survival of *D. siricidicola* on the South African field strain and the Australian laboratory strain of *A. areolatum*, to examine a possible incompatibility between nematode and fungal strain. Secondly, we used laboratory growth and competition assays to examine the competitive interactions of these two *A. areolatum* strains with the two most common sapstain fungi, *Diplodia pinea* and *Ophiostoma ips*, that occur in trees infested with *S. noctilio* in South Africa. The effect of water potential on the outcome of these fungal interactions was also considered. According to the data *D. siricidicola* survives at comparable levels on the two *A. areolatum* strains. Incompatibility between nematode and fungus strain is consequently unlikely to be the main cause of variable success with *D. siricidicola*. Water potential of the media significantly influenced growth of the fungi and their ability to capture primary resource in competitive interactions ($p < 0.0001$). *Amylostereum areolatum* grew more slowly than the sapstain fungi, but was able to defend its resource. At lower water potentials, *D. pinea* grew faster, captured more primary resource and replaced *O. ips*. The results suggest that competitive interactions between *A. areolatum* and sapstain fungi could negatively influence the success of *D. siricidicola*, especially under conditions of lowered water potential.

Introduction

Amylostereum areolatum Boiden is a Basidiomycetes fungus which has a mutualistic symbiotic relationship with siricid woodwasps (Cartwright 1929, Talbot 1977, Slippers *et al.* 2003). This fungus has been recorded in symbioses with the woodwasps *Sirex noctilio*, *S. juvencus*, *S. nitobei* and *S. cyaneus* (Bedding and Ackurst 1978), and more recently with *S. edwarsii* and *S. 'nitidus'* (Nielsen *et al.* 2009). Of these symbioses, the *S. noctilio* – *A. areolatum* association is the best known. This is because this insect is an invasive alien pest on *Pinus* spp. in many southern hemisphere countries where these trees are extensively grown in plantations.

Sirex noctilio attacks various conifer species, but predominantly *Pinus* species (Spradbery and Kirk 1978). *Sirex noctilio* and *A. areolatum* in concert overcome the defences of the host tree and provide an ideal environment for the development of the fungus and wasp larvae (Coutts 1969, Spradbery 1973, Talbot 1977). The fungus benefits by being dispersed by the wasp and introduced into a weakened host, caused by the phytotoxic mucous injected into the tree together with the eggs and fungus. In turn, the fungus is essential for the nutrition of the larvae (Coutts 1969, Spradbery 1973, Talbot 1977).

Sirex noctilio is native to Eurasia, but has been accidentally introduced into many pine growing countries of the southern hemisphere during the course of the twentieth century (Miller and Clarke 1935, Gilbert and Miller 1952, Spradbery and Kirk 1978, Tribe 1995, Maderni 1998, Klasmer *et al.* 1998, Iede *et al.* 1998, Hurley *et al.* 2007). In these countries, the *S. noctilio* – *A. areolatum* complex has resulted in major losses in pine plantations (Haugen 1990, Maderni 1998, Hurley *et al.* 2007). Most recently, *S. noctilio* was detected in North America (Hoebeke *et al.* 2005, de

Groot *et al.* 2007). The threat that the *S. noctilio* – *A. areolatum* complex will pose to pine forests in North America is still uncertain, but Yemshanov *et al.* (2009) estimated the potential damage in Canada over the next 20 years to be as much as $254 million per year.

The nematode *Deladenus* (= *Beddingia*) *siricidicola* Bedding, parasitic to *S. noctilio*, was discovered and described in the 1960s (Bedding 1968). *Deladenus siricidicola* is extraordinary in having both a parasitic and mycetophagous life-cycle (Bedding 1972). In the mycetophagous life-cycle, the nematodes feed on *A. areolatum*. In the vicinity of *S. noctilio* larvae, the high CO_2 and low pH environment stimulates the parasitic life-cycle, where the nematodes parasitize the larvae. The nematodes do not kill the larvae, but develop and reproduce inside the larvae, and sterilize the eggs of the emerging female wasp. Infected female wasps lay nematode-filled eggs in to new trees, thus spreading the nematode (Bedding 1972, Bedding and Iede 2005). *Deladenus siricidicola* feeds exclusively on *A. areolatum* in the mycetophagous life-cycle and can go through many generations in the absence of the parasitic life-cycle (Bedding 1972, Spradbery and Kirk 1978). The mycetophagous life-cycle takes approximately two weeks while the parasitic life-cycle follows the life-cycle of the wasp, which may vary from one to three years depending on the environment (Bedding 1972).

Although complex, the biology of *D. siricidicola* makes it an ideal biological control agent for *S. noctilio* (Bedding and Iede 2005). The nematode can be mass reared on cultures of *A. areolatum* in a short period of time and inoculated into *S. noctilio*-infested trees where they feed on *A. areolatum* in the tree until they locate *S. noctilio* larvae. *Deladenus siricidicola* has been released as a biological control agent in every southern hemisphere country where *S. noctilio* is a pest, often attaining parasitism levels between 70-100 % (Bedding and Akhurst 1974, Iede *et al.* 1998, Tribe and Cillié 2004, Carnegie *et al.* 2005). However, success with *D. siricidicola* has been variable (Hurley *et al.* 2007). In particular, inoculations with the nematode in the summer rainfall region of South Africa resulted in less than 10 % parasitism (Hurley *et al.* 2007).

A possible explanation for the limited success of *D. siricidicola* inoculations is competition between *A. areolatum* and sapstain fungi in the wood (King 1966). This hypothesis is based on the fact that competition for resources between fungi living on the same substrate is well known and involves primary resource capture or combat, where already colonized resources are captured or defended (Cooke and Rayner 1984, Rayner and Webber 1986, Boddy 2000). For the *S. noctilio* system, sapstain fungi are often present in trees infested by this insect (B.P. Hurley, personal observation), and therefore *A. areolatum* and these fungi would need to compete for the same resource. Two sapstain fungi common in *Pinus* sp. in South Africa are *Diplodia pinea* (Desm.) Kickx (formerly *Sphareopsis sapinea* (Fr.) Dyko and Sutton) and *Ophiostoma ips* (Rumb.) Nannf. (Wingfield and Swart 1994, Zhou *et al.* 2001). *Diplodia pinea* is a latent pathogen associated with stress and wounds (Swart and Wingfield 1991), and is commonly found in trees infested with *S. noctilio*. Similarly, *O. ips* is associated with the bark beetle, *Orthotomicus erosus* (Wollaston), which attacks stressed trees (Tribe 1992, Zhou *et al.* 2001) and it is also common in trees infested with *S. noctilio*. The limited spread of *A. areolatum* as a result of competition with these sapstain fungi could decrease the likelihood that the nematodes will survive until they find a fungal source or *S. noctilio* larvae.

Water availability (water potential) can influence the outcome of competitive interactions between fungal species (Shearer 1995, Boddy 2000). Studies on the competitive abilities of insect-associated fungi have shown that the primary and combative ability of the fungi, as well as water potential, influences the outcome of competitive interactions and can have important consequences for the success of the insect – fungus association (Klepzig and Wilkens 1997, Klepzig *et al.* 2004, Bleiker and Six 2009). The influence of moisture availability on inoculation success with *D. siricidicola* was suggested by Hurley *et al.* (2008), who showed that inoculation success was lowest in the drier top section of the tree.

The strain of *A. areolatum* that occurs together with *S. noctilio* is another factor that could influence inoculation success with *D. siricidicola*. Certain strains of *A. areolatum* from the field in Australia were found to be preferable for rearing the nematode to others (R.A. Bedding personal communication in Slippers *et al.* 2001, author, unpublished results). Further, Slippers *et al.* (2001) showed differences in the strain of *A. areolatum* present in South Africa and the strain imported from Australia, which is used to rear the nematode. An incompatibility between nematode and fungal strain could result in reduced nematode reproduction and consequently reduced parasitism of *S. noctilio*. Furthermore, the *A. areolatum* strain in South Africa could compete poorly with sapstain fungi compared to *A. areolatum* strains in other countries.

In this study we tested two hypotheses related to the fungal symbiont of *S. noctilio* that might influence the success of the nematode in biological control programs. Firstly, we compared the survival of different *D. siricidicola* populations on two different strains of *A. areolatum*, to determine if specificity on the fungus can influence fitness of the nematode. Secondly, we examined the competitive ability of the two *A. areolatum* strains with the sapstain fungi *D. pinea* and *O. ips*. Competitive interactions were examined in terms of primary resource capture and combat, and the influence that water potential has on the outcome.

Materials and Methods

Nematode sources and fungal strains

Two strains of *A. areolatum* were used in this study. The Australian laboratory strain (*A. areolatum* AUS) was sent from Australia with cultures of *D. siricidicola* in 2003. This strain has been used for rearing nematodes for field release in South Africa since 2004. The South African field strain (*A. areolatum* KZN) was isolated from the field in 2007 and represents the fungus accidentally introduced with *S. noctilio*. The *D. pinea* strain used was isolated from *P. patula* in Vryheid, South Africa, in 2007. The *O. ips* strain was isolated from galleries of *O. erosus* on a *Pinus* sp., in Lothair, South Africa, in 2009.

All nematodes used in the study were of the Kamona strain of *D. siricidicola* (Bedding and Iede 2005). Laboratory reared cultures from different nematode populations / sources were used, to examine whether the rearing history of the nematode can influence its survival on a specific *A. areolatum* strain. Four different nematode sources were used, namely BRA, ARG, KZN and KZN2. Two of the sources were from laboratory cultures in Brazil (BRA) and Argentina (ARG). The BRA and ARG sources were obtained in 2006 and have been reared on *A. areolatum* (AUS). The other two nematode sources were the original nematodes imported to South Africa from Australia in 2003, introduced into the field, and retrieved from parasitized wasps. The KZN source was retrieved in 2008 and subsequently grown on

A. areolatum (AUS). The KZN2 source was retrieved from the field in 2007 and subsequently grown on *A. areolatum* (KZN).

Nematode survival assays

For the nematode survival assays, Potato Dextrose Agar (PDA) (40 g l^{-1} potato dextrose extract, 15g l^{-1} agar) in 90mm Petri dishes and wheat-rice medium in 500 ml flasks (84 g wheat, 36 g brown rice, 80 ml water) was used as a growth medium for *A. areolatum*. The flasks containing this medium were autoclaved at 121 °C for 30 min, left to stand for 24 hours and then autoclaved again at 134 °C for 15 min. Flask cultures (see Bedding and Iede 2005) were established for each of the four nematode sources, using *A. areolatum* (AUS). After approximately six weeks, nematodes were rinsed three times from each flask using tap water. Three samples were taken from the nematode sediment to estimate the total number of nematodes present. For each sample, one ml was removed from the sediment and diluted in 49 ml of tap water, after which one ml was removed from this 50 ml solution and the total number of nematodes in this sample was counted using a Petri dish with 0.5 cm^2 grids, under a microscope at 20x magnification. The nematode-water solution was agitated before each sample was taken to ensure the solution was well mixed. The average of the three sample counts was used to calculate an estimate for the number of nematodes in the flask.

PDA plates covered with one third of either *A. areolatum* (AUS) or *A. areolatum* (KZN) were used to start the cultures for the nematode assays. Nematodes from each of the different sources were placed on plates with *A. areolatum* (AUS) and plates with *A. areolatum* (KZN). In total there were eight treatments of nematode source and fungal strain combination. Approximately 30000 nematodes were placed on each plate. The fungus was inoculated on one side and the nematodes were placed on the opposite side of the plate. The nematodes moved towards the fungus and after 12 hours two fungus-nematode plugs were cut out and placed face-down on clean PDA plates. After two weeks, three fungus-nematode plugs of approximately the same size were removed from these new cultures and placed in flasks prepared with a wheat-rice medium. A plug of similar size, containing only the fungus was also placed in the flask, to ensure that the nematodes had sufficient food. After six weeks the nematodes were rinsed from the flasks and counted, as described above. For each treatment the 10 flasks which appeared to have the highest number of nematodes were counted. An Analysis of Variance (ANOVA) using a General Linear Model (GLM) (SAS version 8.2 (SAS Institute, 2001)) was used to examine differences in total nematode numbers between the nematode source and fungal strain treatments.

Fungal growth assays

Growth media at different water potentials used for the fungal growth assays was based on previously published methods (Whiting and Rizzo 1999, Klepzig *et al.* 2004). Media were prepared with the osmotic potentials of 0 (unamended malt extract agar (MEA), 15 g l^{-1} agar, 10 g l^{-1} malt extract), -5 megapascals (MPa) (MEA amended with 8.2 g l^{-1} KCl and 68.1 g l^{-1} sucrose) and -10 MPa (MEA amended with 16.6 g l^{-1} KCl and 134.2 g l^{-1} p sucrose) in 90 mm Petri dishes. The growth of *A. areolatum* (AUS), *A. areolatum* (KZN), *D. pinea* and *O. ips* was examined on the three different media. A 0.5 cm diameter disc of fungus was placed face down at the centre of the plates. Mycelial growth was measured every second day from the fourth day after inoculation, for a total of 18 days after inoculation. Plates were sealed with Parafilm and maintained at 20 °C in the dark. Measurements were done using Image Tool v3.00 Microsoft® and the total area covered by the fungus was calculated. An Analysis of Variance (ANOVA) using a General Linear Model (GLM) and a Repeated Measures ANOVA (SAS version 8.2 (SAS Institute, 2001)) was used to analyze the growth of fungi. STKROMME (SHC du Toit, South African Institute for Statistical Research, HSRC, 1980) was used to fit a Gompertz curve to the data.

Fungal competition assays

Competition between *A. areolatum* (AUS), *A. areolatum* (KZN), *D. pinea* and *O. ips* was examined on the same three media as used for the fungal growth assays. A 0.5 cm diameter disc of each fungus in the interaction was placed on opposite sides of the plate. The growth was measured from the fourth day after inoculation and every week after that for a total of nine weeks. The competition assays continued over a longer period of time than the growth assays, to ensure that the fungi had opportunity to compete fully. Plates were sealed with Parafilm and maintained at 20 °C in the dark. Measurements were done using Image Tool v3.00 Microsoft® and the total area covered by the fungus was calculated. An Analysis of Variance (ANOVA) using a General Linear Model (GLM) and a Repeated Measures ANOVA (SAS version 8.2 (SAS Institute, 2001)) was used to analyze the growth of fungi. STKROMME (SHC du Toit, South African Institute for Statistical Research, HSRC, 1980) was used to fit a Gompertz curve to the data.

Results

Nematode survival assays

The number of nematodes produced per flask ranged from 170000 to 4.3 million, with an average of 1.3 million. Considering all nematode sources together, significantly more nematodes were produced on flasks with *A. areolatum* (AUS) (Least Squares (LS) mean = 1.5 mil.) than on flasks with *A. areolatum* (KZN) (LS mean = 1.1 mil.) ($p = 0.0037$). This difference was mainly due to the highly significant difference in numbers of the BRA nematode source on *A. areolatum* (AUS) (LS mean = 2 mil) and *A. areolatum* (KZN) (LS mean = 840 000) (Table 1). None of the other nematode sources showed significant differences in numbers produced on the two *A. areolatum* strains (Table 1). The number of nematodes produced for the different nematode sources was not significantly different, except of the ARG nematode source, which produced significantly less than the other sources ($p = 0.0094$). None of the nematode sources produced significantly more nematodes on *A. areolatum* (KZN), compared to the KZN nematode source (Table 1).

Fungal growth assays

The growth of the fungi was significantly influenced by time, medium, the fungus species / strain and the interaction between medium and fungus species / strain ($p < 0.0001$). Growth of all four fungi decreased with decreasing water potential (Fig. 1). *Amylostereum areolatum* (KZN) grew significantly faster than *A. areolatum* (AUS) on MEA ($p < 0.05$), but not on -5 MPa and -10 MPa (Fig. 1). *Diplodia pinea* and *O. ips* grew significantly faster than *A. areolatum* (AUS) and *A. areolatum* (KZN) on MEA and -5 MPa ($p < 0.0001$) (Fig. 1). *Diplodia pinea* grew faster than *A. areolatum* (AUS), *A. areolatum* (KZN) and *O. ips* on -10 MPa ($p < 0.05$) (Fig. 1). The growth of *D. pinea* and *O. ips* slowed down and stopped as it came close to the edge of the Petri dishes.

Fungal competition assays

The growth of the fungi in the competition assays was significantly influenced by time and medium ($p > 0.0001$). There was no clear difference in the competitive interaction of *A. areolatum* (AUS) and *A. areolatum* (KZN) with the two sapstain fungi, *D. pinea* and *O. ips* (Fig. 2). *Diplodia pinea* captured more primary resource than *A. areolatum* on all media, but competed increasingly better against *A. areolatum* with decreasing water potential (Fig. 2). Despite the great differences in primary resource capture, *D. pinea* did not replace *A. areolatum* on any medium. A barrier was formed between the two fungi on MEA and -5 MPa, with inhibition of hyphal growth at a distance, resulting in a deadlock between the fungi (Fig 3A-B). On -10 MPa, the growth form of *D. pinea* changed and hyphal growth appeared to stop at the point of contact between the two fungi (Fig 3C).

The competitive ability of *O. ips* against *A. areolatum* decreased with a decrease in water potential (Fig. 2). On MEA, *O. ips* captured far more primary resource than *A. areolatum* (Fig 2, Fig 3D). After four weeks a barrier was formed between the fungi, with inhibition at a distance. *Ophiostoma ips* also captured more primary resource at -5 MPa, but the difference in growth between the two fungi was not as great, and no barrier was observed for the duration of the study (Fig 3E).

Amylostereum areolatum captured more primary resource than *O. ips* on -10 MPa (Fig. 2). At -10 MPa, isolates of *O. ips* changed in morphology (Fig 3F).

The competitive ability of *O. ips* against *D. pinea* decreased with a decrease in water potential (Fig. 2). On MEA, *O. ips* initially captured more primary resource than *D. pinea*. A barrier was formed after three weeks, which inhibited the growth of *O. ips* (Fig 3G). *Diplodia pinea* continued to grow, but at the end of the study there appeared to be a deadlock between the two fungi. This deadlock situation only occurred in some of the interactions between *D. pinea* and *O. ips* and was thus not evident in Fig. 2 which represented the average growth. On -5 MPa and -10 MPa, *D. pinea* captured more primary resource and replaced *O. ips* (secondary resource capture) (Fig 2, Fig 3H-I).

Discussion

Results of the study showed that the *D. siricidicola* released in South Africa as a biological control agent against *S. noctilio* is able to survive and reproduce on the South African field strain of *A. areolatum*, introduced naturally with *S. noctilio*, at levels comparable to its survival on the genetically different Australian strain (Slippers *et al.* 2001), used to raise the nematodes. The survival and reproduction of the nematode was also comparable to that of the nematode strain that has been introduced in Brazil and Argentina. The nematodes released in Brazil and Argentina have given parasitism levels of over 70 % in their respective countries (Iede *et al.* 1998, V. Klasmer, personal communication), where they are associated in the field with the same strain of *A. areolatum* introduced into South Africa with *S. noctilio* (Slippers *et al.* 2001). The comparable survival of all the nematode sources used for biological control in southern hemisphere countries on the strain of *A. areolatum* occurring with the wasp under field situations in South Africa and South America indicates that incompatibility between *A. areolatum* (KZN) and the Kamona strain of *D. siricidicola* is an unlikely explanation for the low inoculation success in the summer rainfall areas of South Africa.

The KZN2 nematodes reared on *A. areolatum* KZN for multiple generations did not give rise to higher numbers of nematodes on *A. areolatum* KZN in this study. This suggests that efforts to breed for greater survival on the *A. areolatum* (KZN) fungus using the Kamona strain of *D. siricidicola* are unlikely to be successful. It is not clear why the nematodes sourced from Brazil gave rise to significantly higher numbers on *A. areolatum* (AUS) than on *A. areolatum* (KZN), but this result highlights the possibility of increased fitness of specific nematode strains on specific fungal strains. Further investigations to understand the behaviour of nematode strains in different environments, as well as their comparable fitness on different *A. areolatum* strains are clearly warranted.

Amylostereum areolatum generally grew more slowly than the sapstain fungi *D. pinea* and *O. ips*. However, the *S. noctilio* symbiont showed strong defence capabilities and was never replaced by the sapstain fungi. *Amylostereum areolatum* defended its food resource through the formation of an antagonistic barrier zone some distance from the competing fungi. Alternatively, the fungus produced a stationary barrier zone of mycelium resistant to invasion, at the point of mycelial contact. These forms of combative interactions are well known in fungi. Boddy (2000) stated that antagonism at a distance, hyphal interference, mycoparasitism and gross mycelial contact were the main forms of combative interactions. The outcomes of these interactions may result in replacement of one fungus with another or a deadlock between the two species, as observed between *A. areolatum* and the sapstain fungi.

The growth rate and competitive ability of the two *A. areolatum* strains in this study were very similar on most media. There were only differences on the media with the highest water potential (MEA), where the South African strain grew faster. Inoculation success of *D. siricidicola* is particularly poor at low moisture availability (Hurley *et al.* 2008), but there were no significant differences in competitive ability of the two *A. areolatum* strains on media with lower water potentials. These results indicate that the difference in the competitive ability of the two *A. areolatum* strains is unlikely to explain the low levels of inoculation success in the summer rainfall areas of South Africa.

Reduced water potential decreased the growth of all of the fungi considered in this study, including the *S. noctilio* symbiont, *A. areolatum*, and the sapstain fungi that it encounters in woodwasp-infested trees. For *A. areolatum*, this is consistent with the experience of Boddy (1983) who noted that wood decay Basidiomycetes generally do not grow well below -4.4 MPa. Although *A. areolatum* growth decreased at lower water potentials, the fungus did not cease to grow and was able to compete for resources with the sapstain fungi. In contrast to *A. areolatum*, *D. pinea* displayed its best growth on the media having low water potential and this is consistent with the fact that some Ascomycetes are favoured by low water potentials (Boddy 2000). However, *O. ips* showed very poor growth and competitive ability at low water potentials, to such an extent that it was replaced by *D. pinea*. This result is similar to that of Klepzig *et al.* (2004) who found that the related *Ophiostoma* sp., *Ophiostoma minus* (Hedgcock) H. and P. Sydow had decreased competitive ability on media having low water potential.

Mycelial morphology of fungi can change during competitive interactions (Rayner and Webber 1986, Boddy 2000), and this was seen in the present study. For example, the growth of *D. pinea* was enhanced in the competitive interactions, sometimes resulting in a change in the mycelial morphology and directed growth towards the competing fungus. This was especially noticeable on media having reduced water potential. *Ophiostoma ips* also showed changes in mycelial morphology on the low water potential media. In this case, the mycelial morphology changed to a less dense and more rapid growth. Boddy (2000) referred to these changes in mycelial morphology as ‘phase shifts’, which can result in a redistribution of the mycelium, but also make a fungus more susceptible to invasion if the interfacial zone is breached (Boddy 2000).

The growth and competitive ability of the fungi as was seen in this study offer important insights into factors that might affect the establishment of *A. areolatum* in the field and consequently the survival of *D. siricidicola*. *Amylostereum areolatum* is likely to be able to grow in the low moisture conditions that the nematodes experience in the summer rainfall area of South Africa (Hurley *et al.* 2008). The growth under these conditions would be slow, and most of the resource could then be captured by *D. pinea*, which is uniformly present in *Pinus* spp. in South Africa (Swart *et al*. 1985, Wingfield and Swart 1994). However, *A. areolatum* would be able to defend its resource, thus securing the food source of the *S. noctilio* larvae and *D. siricidicola*, which would have entered the tree naturally with *A. areolatum* and having followed its growth.

Artificial inoculations with *D. siricidicola* occur some time after the tree is attacked by *S. noctilio*, as symptoms of attack must first be observed (Haugen *et al*. 1990). These trees would have already lost moisture since the attack and at that time

D. pinea infection is likely to be well established. This could decrease the survival of the inoculated nematodes in the tree, as they still need to locate the areas of *A. areolatum* establishment. *Amylostereum areolatum* that is introduced together with nematodes during inoculation is unlikely to be able to compete with *D. pinea* and other fungi that are already established.

The suggestion that competitive interactions with *A. areolatum* could have greater significance on inoculation success than natural parasitism of *D. siricidicola* is supported by other data. Although high levels of parasitism have been obtained in South America, results have been variable, and Hurley *et al.* (2007) suggested that inoculation success is low, but that natural parasitism levels can be high. Similarly, inoculations in the Cape province of South Africa originally gave poor results (22.6 %), but natural spread of the nematode increased parasitism to 96.1 % just two years later (Tribe and Cillié 2004). Recent data from the summer rainfall areas also indicates that natural parasitism is increasing (above 50 % in some sites, in the bottom section of the trees), although inoculation success still remains poor (Brett Hurley, unpublished data)

The results of laboratory growth and competition assays with fungi, such as those obtained in this study, should be interpreted with caution. While they might reflect the field environment, this may not always be the case. For example, the outcome of interactions in the natural environment can be influenced by the microclimate, the size and quality of the resource, temperature, and the presence of multiple competing fungi (Boddy 2000). In our study we examined the influence of one variable, water potential, but other variables are likely to also influence the outcome of the interactions studied. While it would be difficult to consider the interactions of different fungi and nematodes in wood and under natural conditions, such studies could provide further insight in to the establishment of *A. areolatum* in the field.

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Table 1. P-value table for nematodes produces on *A. areolatum* AUS and *A. areolatum* KZN fungus, on four nematode sources (ARG, BRA, KZN, KZN2). The difference in nematode numbers between treatments was not significantly different (NS), significantly different where p < 0.05 (*), significantly different where p < 0.01 (**), or highly significantly different where p < 0.001 (***).

		A. areolatum AUS				*A. areolatum* KZN			
		ARG	BRA	KZN	KZN2	ARG	BRA	KZN	KZN2
A. areolatum AUS	ARG	-	**	NS	*	NS	NS	NS	NS
	BRA		-	*	NS	***	***	*	NS
	KZN			-	NS	*	NS	NS	NS
	KZN2				-	***	**	NS	NS
A. areolatum KZN	ARG					-	NS	*	**
	BRA						-	NS	*
	KZN							-	NS
	KZN2								-

Figure 1. Comparison of the cumulative growth of four fungi, *A. areolatum* AUS, *A. areolatum* KZN, *D. pinea* and *O. ips*, on three media of different water potential, over an 18 day period. Growth curves that did not fit a Gompertz function are indicated with an asterix (*).

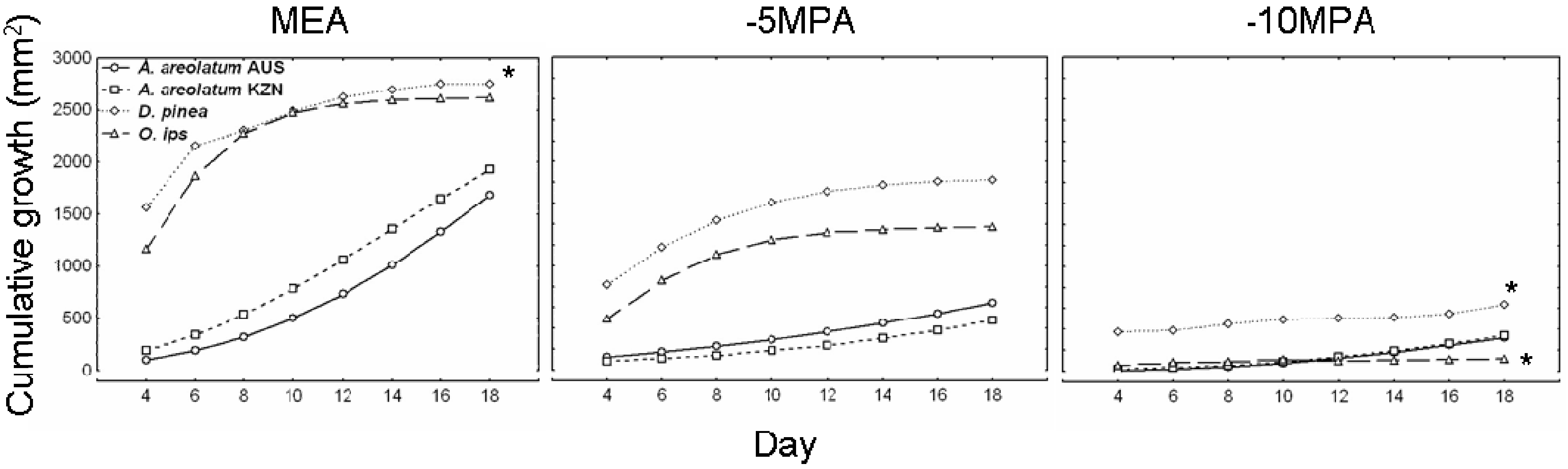

MEA
-5MPA
-10MPA
Cumulative growth (mm²)
3000
2500
2000
1500
1000
500
0
A. areolatum AUS
A. areolatum KZN
D. pinea
O. ips
*
*
*
4
6
8
10
12
14
16
18
Day

Figure 2. Comparison of the cumulative growth of four fungi, *A. areolatum* AUS, *A. areolatum* KZN, *D. pinea* and *O. ips*, competing with each other on three media of different water potential, over a nine week period. Growth curves that did not fit a Gompertz function are indicated with an asterix (*).

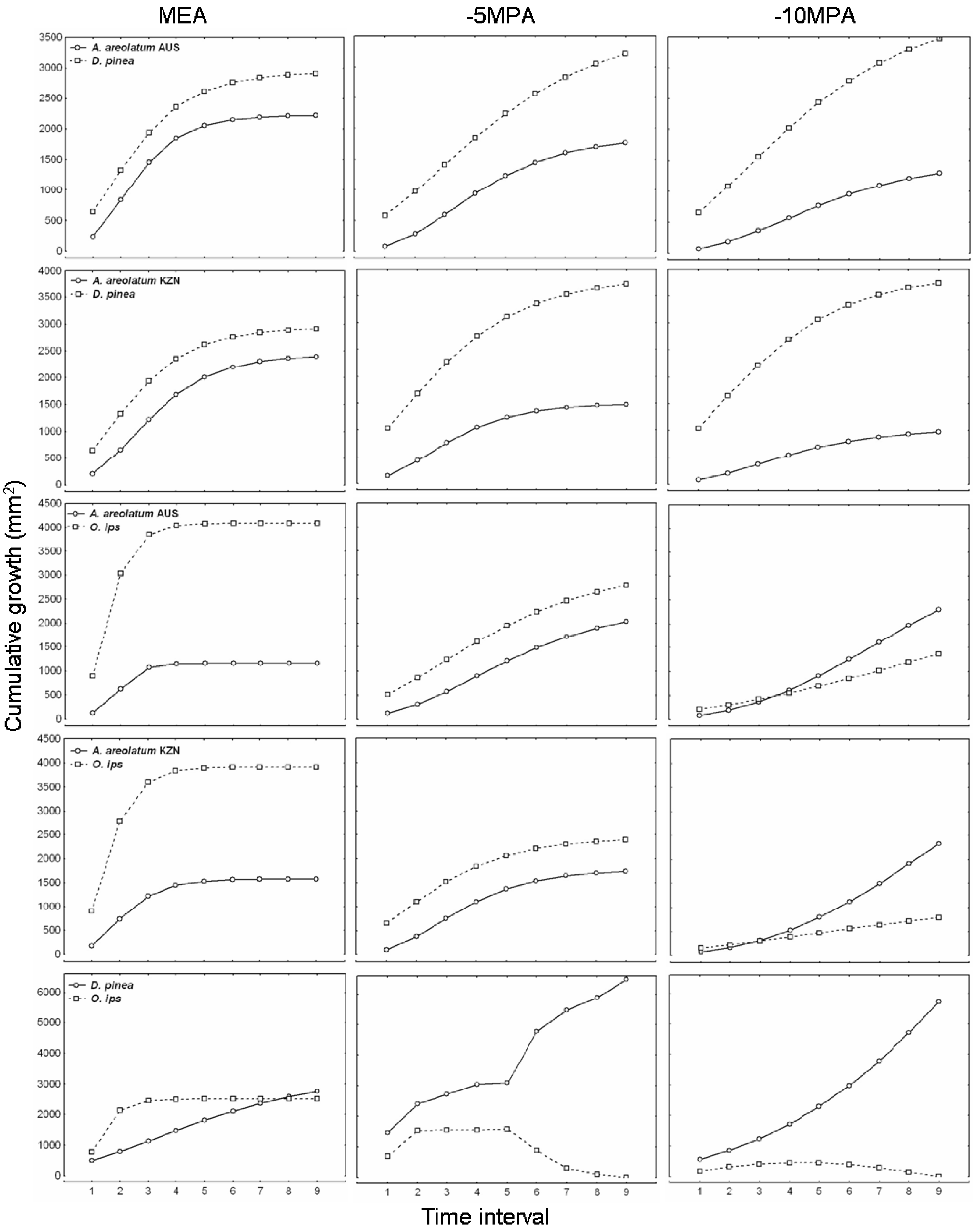
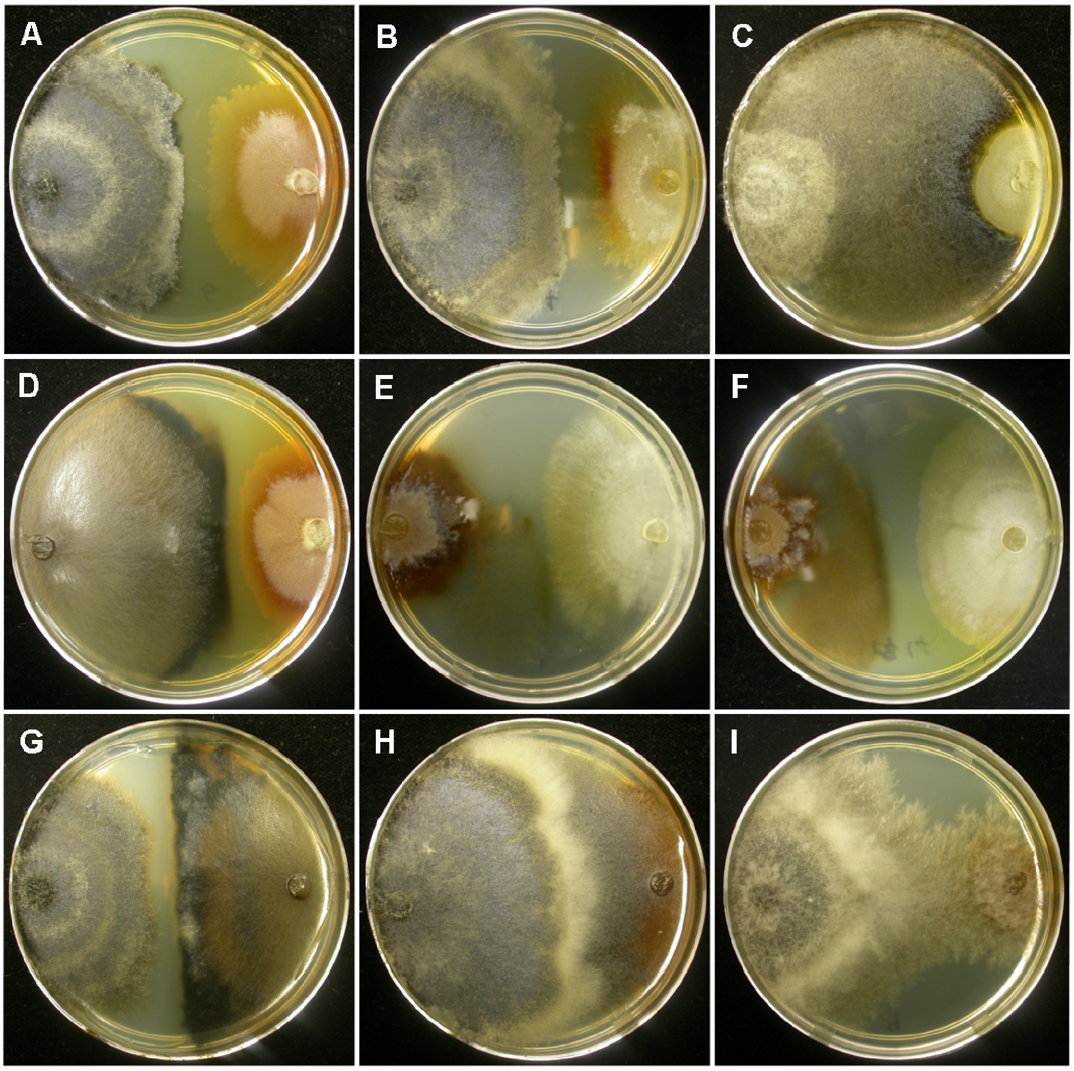

MEA
-5MPA
-10MPA
Cumulative growth (mm^2)
Time interval
A. areolatum AUS
D. pinea
A. areolatum KZN
D. pinea
A. areolatum AUS
O. ips
A. areolatum KZN
O. ips
D. pinea
O. ips
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
6000
1
2
3
4
5
6
7
8
9

Figure 3. Examples of competitive interactions between the fungi *A. areolatum*, *D. pinea* and *O. ips*. A-C = *D. pinea* (left) competing with *A. areolatum* (right) on MEA, -5MPa and -10MPa medium, respectively. D-F = *O. ips* (left) competing with *A. areolatum* (right) on MEA, -5MPa and -10MPa medium, respectively. G-I = *D. pinea* (left) competing with *O. ips* (right) on MEA, -5MPa and -10MPa medium, respectively. Pictures were taken at week seven.


A
B
C
D
E
F
G
H
I

CHAPTER FOUR

Sequence data reflect the introduction history of the Sirex woodwasp parasitoid, *Ibalia leucospoides* (Ibaliidae, Hymenoptera)

The parasitoid wasp *Ibalia leucospoides* native to the northern hemisphere, has been introduced to the southern hemisphere as a biological control agent for the invasive woodwasp *Sirex noctilio*. Two sub-species of the parasitoid, *I. leucospoides leucospoides* (Palearctic distribution) and *I. leucospoides ensiger* (Nearctic distribution), were introduced and have subsequently hybridized. Despite extensive records of the numbers and origins of the wasps imported into the southern hemisphere, nothing is known regarding the current population diversity. In this study, the genetic variation of *I. leucospoides* in its native and introduced ranges was examined using mitochondrial (cytochrome oxidase subunit one (COI)) and nuclear (internal transcribed spacer (ITS)) markers. As expected, mitochondrial DNA diversity was higher in the native range. Introduced populations had fewer clades separated by deep divergence and greater nuclear ITS diversity than the native North American population examined. Incongruence in the clades for the ITS and mtDNA trees suggests that diversity in the southern hemisphere is intraspecific, possibly due to hybridization. The results reflect the extensive introductions of *I. leucospoides* from four continents and including two sub-species and hundreds of wasps, followed by genetic bottlenecks that fixed only a few haplotypes in these populations. The data further suggest that *I. leucospoides* populations in South America and South Africa originated in New Zealand or Australia.

Introduction

Ibalia leucospoides Hochenw. (Ibaliidae, Cynipoidea, Hymenoptera) is a parasite of siricid woodwasps (Chrystal and Myers 1928, Spradbery and Kirk 1978, Norlander *et al.* 1996), which owes its notoriety to the fact that it has been widely used for biological control of the invasive woodwasp *Sirex noctilio* F. in the southern hemisphere. The female *I. leucospoides* uses the oviposition hole made by siricid females to insert its eggs into those of the siricid host or its early instar larvae (Flanders 1925). Interestingly, *I. leucospoides* locates its siricid host by using volatiles released by the fungal symbiont of the woodwasp (Madden 1968, Martinez *et al.* 2006).

There are 19 species of Ibaliidae (Norlander *et al.* 1996). Kerrich (1973) revised the genus *Ibalia* and reduced *I. ensiger* Norton in North America and *I. suprunenkoi* Jacobson in Japan, to synonymy with *I. leucospoides*. Kerrich (1973), however, recognized the sub-species *I. leucospoides leucospoides* with a Palaearctic distribution and *I. leucospoides ensiger* with a Nearctic distribution.

Ibalia leucospoides is considered one of the most successful parasitic wasps introduced into the southern hemisphere for the control of the woodwasp *S. noctilio* (Neumann *et al.* 1987, Iede *et al.* 2000, Carnegie *et al.* 2005, Hurley *et al.* 2007). *Sirex noctilio* is native to Eurasia, but was accidentally introduced to the southern hemisphere during the course of the twentieth century (Miller and Clarke 1935, Gilbert and Miller 1952, Spradbery and Kirk 1978, Tribe 1995, Maderni 1998, Klasmer *et al.* 1998, Iede *et al.* 1998, Hurley *et al.* 2007). The pest has spread widely in the southern hemisphere and is now found in New Zealand, Australia, Uruguay, Argentina, Brazil, Chile and South Africa. It has also recently appeared as a non-native invasive pest in North America (Hoebeke *et al.* 2005, de Groot *et al.* 2007). *Sirex noctilio*, together with its fungal symbiont *Amylostereum areolatum* Boiden, attacks and kills pine trees (Talbot 1977). Although not a pest in its native range, *S. noctilio* has become a serious pest in its introduced range in the southern hemisphere (Haugen 1990, Maderni 1998, Hurley *et al.* 2007). This has prompted the introduction of parasitic nematodes and wasps, including *I. leucospoides*, from the northern hemisphere as biological control agents (Taylor 1976, Bedding and Iede 2005, Hurley *et al.* 2007).

Both *I. leucospoides leucospoides* and *I. leucospoides ensiger* were introduced to the southern hemisphere as biological control agents. The first attempt to introduce *I. leucospoides leucospoides* was from England to New Zealand in 1931 (Nuttall 1989). This attempt failed, but later introductions from England in 1950-1951 were successful, and resulted in a breeding colony in New Zealand. In 1959-1960 *I. leucospoides leucospoides* was sent from New Zealand to Tasmania (Taylor 1967). Further introductions of *I. leucospoides leucospoides* from Europe, Turkey, Morocco and Japan, and introductions of *I. leucospoides ensiger* from USA and Canada, to Tasmania occurred from 1962-1973 (Taylor 1976). *Ibalia leucospoides ensiger* was sent from Tasmania to New Zealand and both *I. leucospoides* sub-species were sent from Tasmania to mainland Australia (Taylor 1976, Nuttall 1989). Interestingly, *I. leucospoides leucospoides* and *I. leucospoides ensiger* were reported to interbreed, with the hybrids being indistinguishable from the parent sub-species (Nuttall 1989).

In South America, *I. leucospoides* was reported as naturally introduced with *S. noctilio* and its origin was thus not known (Eskiviski *et al.* 2004). It was first detected in Uruguay in 1984 and subsequently spread to Argentina, Chile and Brazil. In 1998, *I. leucospoides* was imported into South Africa from Uruguay (Tribe and Cillié 2004).

It is not known with certainty which sub-species of *I. leucospoides* were introduced into South America, and subsequently into South Africa. *Ibalia leucospoides ensiger* together with other native siricid parasitoids was already present in North America when *S. noctilio* was first detected there (Cameron 1965, Liu and Nordlander 1992, Smith and Schiff 2002).

Almost nothing is known regarding the genetic variation within and between populations of *I. leucospoides* (Hurley *et al.* 2007). Collections of *I. leucospoides* for release in New Zealand and Australia were from a wide geographical area and involved relatively large numbers of wasps (Taylor 1967, 1976, Nuttall 1989). The origin of *I. leucospoides* introduction into South America is unknown and it is also not known how many introductions occurred. The introduction of *I. leucospoides* from Uruguay to South Africa was based on a very small number of wasps (Tribe and Cillié 2004, Hurley *et al.* 2007) which may have resulted in little or no genetic variation in this population.

The aim of this study was to consider the genetic diversity of *I. leucospoides* across the greater part of its introduced range. For comparison and based on availability, this diversity was compared with a small population from part of the natural range of the parasitoid. For this purpose sequence data for a portion of the mitochondrial cytochrome oxidase subunit one (COI) and the nuclear internal transcribed spacer (ITS) ribosomal DNA were used.

Materials and Methods

Samples and DNA extraction

Ibalia leucospoides adults were obtained from Australia, South Africa, Argentina and Chile, representing much of the introduced range of the wasp in the southern hemisphere (Table 1). Collections of the parasitoid from its native range were difficult to obtain, but included *I. leucospoides ensiger* from the USA and Canada, and two specimens of *I. leucospoides leucospoides* from Portugal (Table 1). Only two specimens were obtained from Portugal. The insect is not commonly encountered in Europe, and we were not able to obtain more wasps from this region. The majority of wasps were collected between 2006 and 2009 and stored in 70-96 % ethanol. The two specimens from Portugal were dry pinned specimens collected in 2001.

Tissue was removed from the thorax of each wasp and total genomic DNA was extracted using the PrepMan™ Ultra Sample Preparation Reagent Protocol (Applied Biosystems, USA), with 100 µl of PrepMan™ Ultra Sample Preparation Reagent used per wasp. For the two samples from Portugal, one to two legs were used and total genomic DNA was extracted using prepGEM™ insect extraction kit (ZyGem Corporation Limited, New Zealand).

Polymerase chain reaction and sequencing

The primers LCO1490 (Folmer *et al.* 1994) and C1-N-2191 (Simon *et al.* 1994) were used to amplify a portion of the mitochondria COI region. The primers

CAS18sF1 and CAS5p8sB1d (the later primer was specific for Hymenoptera) were used to amplify a portion of the nuclear ITS region that covers the ITS1 region between the 18S and 5.8S gene (Ji *et al.* 2003). The COI and ITS primers used did not amplify DNA from the dry specimens from Portugal. Thus, primers were designed using CLC Main Workbench v5.5 to amplify shorter portions within the desired DNA segment. The primer pairs DA2F (5' GGGAAACGTTTTGAGAGA) and DA2R (5' GTATGTAGGAGGAACTATGA), DA3F (5' CTTGTGACTTGTATGCGA) and DA3R (5 TTTCACGATACGGTCCTT), and DA4F (5' CGTTTTGAATGAGCCTGTG) and DA4R (5' TGCGACATCGGCAAAGAA), successfully amplified shorter portions of the ITS segment. The shorter fragments were aligned using overlapping ends to obtain one sequence per specimen. Primers could not be designed within the desired COI region, as this region was inordinately AT rich and had many variable sites. Consequently, a COI sequence was not obtained for the two Portugal specimens.

PCR reaction mixtures contained final concentrations of 1-4 µl of genomic DNA, 10 x PCR buffer (Roche Diagnostics), 0.5 mM of each dNTP, 3.5 mM $MgCl_2$, 1 U *Taq* polymerase, and 0.2 mM of each primer, and were made up with SABAX water to reach a volume of 25 µl. The PCR cycling regime for COI was 95 °C for 7 min, followed by 35 cycles of 95 °C for 45 s, 52 °C for 45 s and 72 °C for 45 s, and concluding with elongation at 72 °C for 10 min. The PCR cycling regime for ITS was 94 °C for 4 min, followed by 35 cycles of 95 °C for 20 s, 62 °C for 40 s and 72 °C for 20 s, and concluding with elongation at 72 °C for 2 min. PCR products were cleaned by precipitation in 3 M NaOAc (pH 4.6) and ethanol. Sequencing reactions were preformed using standard protocols and the products were cleaned using the above-mentioned precipitation method. The ABI Prism^{TM} 3100 Genetic Analyzer (Applied Biosystems) was used for sequencing.

Analyses

Sequences were edited with CLC Main Workbench v5.5 and aligned with Clustal X (Thompson *et al.* 1997). Diversity estimates within localities and sequence divergence values were calculated using DNASP v4 (Rozas *et al.* 2003). TCS (Clement *et al.* 2000) was used to produce a network showing the relationship between haplotypes. Maximum likelihood trees were made with PhyLM 3.0 (Guindon and Gascuel 2003) with a bootstrap analysis of 1000 replications, and using jModelTest v0.1.1 (Posada 2008) to determine the best fit model of nucleotide substitution.

Results

For the COI region, 133 sequences of 661 bp were obtained. For the ITS region, 101 sequences of 777 bp and two sequences of 619 bp (Portugal specimens) were obtained. Due to a limited number of sequences obtained from this area, sequence data for the Chilean and Argentinean samples were combined to represent a South American population. Similarly, sequence data for the USA samples were combined with those for the Canadian specimens to represent a North American population.

Estimates of the COI data showed that the native population of *I. leucospoides ensiger* in North America had greater haplotype diversity than the introduced populations of *I. leucospoides* in the southern hemisphere (Table 2). Fifteen haplotypes were present in the North American population, compared to one to three haplotypes in the southern hemisphere populations. However, the nucleotide diversity of the Australian and South African population was comparable to that of the North American population (Table 2). Only one mitochondrial haplotype was present in the South American population.

The diversity estimates of the ITS data showed more diversity in the southern hemisphere populations than in the native North American and European samples (Table 2). There was only one ITS haplotype in the North American population, compared to two to three haplotypes in each of the southern hemisphere populations. The two specimens from Portugal were identical in their ITS sequence and represented a haplotype different to those in the other populations.

A haplotype network was constructed to show the relationship between the haplotypes (Fig. 1). The North American haplotypes are clearly separate from the southern hemisphere haplotypes. Only one haplotype was represented in more than one region, namely Australia, South Africa and South America. The haplotypes were divided into four groups based on nucleotide differences evident in the haplotype network and also in the clades emerging from the maximum likelihood tree based on the COI data (Fig 2). The highest level of sequence divergence (2.8 %) was between haplotype groups three and four, while the lowest sequence divergence (0.2 %) was between groups one and two (Table 3).

The maximum likelihood trees for the COI and ITS data were compared to display the four mitochondrial haplotype groups and to show where the samples from these groups were represented in the ITS clades (Fig. 3). Thus, samples residing in haplotype group one and two were present in the two southern hemisphere ITS clades, and in the sequences that didn't form a definite clade. Samples residing in haplotype group three were present only in the ITS clade that included samples from Australia, South Africa and South America. Samples residing in haplotype group four were present only in the ITS clade including samples from North America. The two samples from Portugal, representing *I. leucospoides leucospoides* resided in a separate clade.

Discussion

Results of this study based on mitochondrial and nuclear DNA markers generally reflect the introduction histories of populations of *I. leucospoides* used in biological control programmes for *S. noctilio* in southern hemisphere countries. This includes data supporting an extensive introduction programme from different continents and the effects of genetic bottlenecks. Evidence of gene flow, possibly reflecting hybridization of the two *I. leucospoides* sub-species is also shown.

The mitochondrial marker showed greater diversity in a native population of *I. leucospoides* as compared to introduced populations. Mitochondrial DNA is subject to strong genetic drift because of its maternal and haploid mode of inheritance (Avise 2000). Consequently, although many mitochondrial haplotypes of *I. leucospoides* could have been introduced from its native range in to the southern hemisphere, only the dominant haplotypes would likely be retained. In addition, the samples from Australia used in this study were only from New South Wales and parasitoids of *S. noctilio* are known to have been sent from Tasmania to Victoria and then released in to New South Wales from Victoria (Taylor 1976, Carnegie *et al.* 2005). It is possible that the introductions of *I. leucospoides* into New South Wales contained only a portion of the genetic diversity originally introduced into Tasmania and New Zealand. Samples obtained from Tasmania and New Zealand might reveal greater diversity in the introduced range.

Although introduced populations in the southern hemisphere contained only a few mitochondrial haplotypes, the divergence between these haplotypes was deep compared to the native *I. leucospoides ensiger* population. This is shown by the comparable nucleotide diversity between these populations despite having fewer mitochondrial haplotypes in Australia and South Africa (0.00298 and 0.00322 respectively), compared to those in North America (0.00360). The deep divergence between mitochondrial haplotypes in the introduced range reflects the extensive introduction campaign to promote the biological control of *S. noctilio* (Neumann *et al.* 1987). *Ibalia leucospoides* introductions to Australia and New Zealand were from four continents, contained two sub-species and involved hundreds of wasps (Taylor 1967, 1976, Nuttall 1989). The diverse original populations of *I. leucospoides* introduced would be expected to contain divergent mitochondrial sequences due to historic geographic separation of these populations, and this divergence is still represented in the southern hemisphere populations.

The occurrence of a common mitochondrial haplotype in Australia, South Africa and South America reveals the possible origin of *S. noctilio* in South America. If *I. leucospoides* was accidentally introduced into South America together with *S. noctilio* as has been reported (Eskiviski *et al.* 2004), then the source of introduction is most likely Australia, or possibly New Zealand, as *I. leucospoides* has been moved between the two countries. This supports the findings of Slippers *et al.* (2002), where RFLP and nuclear DNA sequence data of the *S. noctilio* fungal symbiont, *A.*

areolatum, showed that *S. noctilio* most likely spread from Australia to South America and later to South Africa.

The presence of three mitochondrial haplotypes in South Africa was surprising given the limited introduction of *I. leucospoides* into this country. Only 18 female *I. leucospoides* were introduced to South Africa from Uruguay (Tribe and Cillié 2004). This indicates that Uruguay has at least three mitochondrial haplotypes of *I. leucospoides*. This is also more than the single haplotype found in the Chilean and Argentinean samples used in this study. *Sirex noctilio* and *I. leucospoides* were first reported in South America in Uruguay (Maderni 1998). It would thus be expected that the genetic diversity of *S. noctilio* and *I. leucospoides* would be higher in this country, compared to subsequently invaded regions. It is also possible that the two mitochondrial haplotypes that are present in Uruguay and not found in Chile and Argentina are present in the latter countries but that they are at low frequencies and were thus not detected in this study.

The ITS rDNA sequences representing a nuclear marker in this study showed greater diversity for *I. leucospoides* in the introduced range than in the native population, where no diversity was present. This is distinctly different from the results obtained using the mitochondrial sequences and could be due to the slower rate of evolution of the nuclear marker (Lin and Danforth 2004). Alternatively, these contradictory results might result from the fact that non-recombinant mitochondrial haplotypes are more easily retained than nuclear haplotypes, which are exposed to homogenization by gene flow (Yang and Kenagy 2009). Either way, the nuclear diversity in the southern hemisphere is expected to reflect the diversity from the different countries of the original populations, while the native population in our study is only represented by one region (eastern Canada). Differing diversity estimates between data from nuclear and mitochondrial sequences are not uncommon. For example, Yang and Kenagy (2009), in their study on deer mice, reported that only 1.4 % of nuclear microsatellite variation was explained by mitochondrial haplotype identity.

The sequence divergence between the mitochondrial haplotype groups (0.2-2.9 %) falls within that expected for intraspecific variation, but the upper level has also been observed in some cases for interspecific diversity. Cognato (2006) reported intraspecific sequence divergence for Hymenoptera to range between 0.6-4.0 %, and between species divergence for Hymenoptera to range between 1.0-9.6 %. Unfortunately, it was not possible to obtain significant numbers of specimens of *I. leucospoides leucospoides* from Europe and there were none from Asia in this study. These data were thus insufficient to fully explore the presence and distribution of the two *I. leucospoides* sub-species and their hybrids in the introduced range. However, the incongruence between clades in trees drawn from the ITS locus compared to clades from mitochondrial sequence data, shows that there has been gene flow between populations representing even the most divergent mitochondrial clades. These results support the hypothesis that the observed mtDNA diversity is intraspecific, or alternatively represents hybrids, as was suggested previously (Nuttall 1989). Hybridization can result in greater invasion success and population vigour (Facon *et al.* 2005, Fitzpatrick and Shaffer 2007) and it would be interesting to examine the comparative spread of the two sub-species and their hybrids if more specimens of *I. leucospoides leucospoides* become available.

Results of this study illustrate the strong effect that genetic drift can have during bottlenecks imposed by importation and initial quarantine rearing of biological control agents. It also shows why mtDNA markers provide a powerful tool to reflect demographic changes and relationships in insect populations. Despite the fact that large numbers of *I. leucospoides* were introduced into rearing programmes in Australasia, only five haplotypes were observed in these populations. These are, however, widely divergent, reflecting the wide geographic region from which *I. leucospoides* was introduced. These findings highlight the importance of considering the potential effect of such bottlenecks on the diversity in biological control populations and their potential to adapt to variable pest populations and environments.

Genetic diversity can influence an organism's ability to colonize and adapt to new environments and host types (Roderick and Navajas 2003, Crawford and Whitney 2010). Introduction of further diversity of *I. leucospoides* could be especially important if new strains of *S. noctilio* are introduced and as *S. noctilio* spreads through regions with variable environmental conditions. It is thus important to better understand the genetic diversity of *I. leucospoides*, as this could influence its present and future success.

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Table 1. Collection locations, number of samples and sample codes used in this study, showing mitochondrial haplotype for each location.

Country	Location	No. samples	Sample code	Mitochondrial haplotype/s
South Africa	Cape Town, Western Cape	14	AA, AB, AD	1, 2, 5
	Stellenbosch, Western Cape	1	AC	5
	Mixed locations, Western Cape	13	AE	1, 5
	Knysna, Western Cape	6	AF	1
	Wellington, Western Cape	2	AG	5
	Boston, Kwa-Zulu Natal	4	BA, BB, BC	1, 5
Chile	unknown	1	CA	1
Argentina	El Bolson	15	CB	1
Australia	Greenhills SF, New South Wales (NSW)	19	DA	1, 3
	Margle SF, NSW	2	DB	1
	Riamu, NSW	3	DC	1
	Nowendoc, NSW	1	DD	1
	Pennsylvania, NSW	9	DE	1, 4
	Green Hills, NSW	2	DG	1
USA	Oswego, New York	2	EA	7, 20
	Syracruse, New York	3	EB	6, 7
Canada	Linda work area, Ontario	6	EC, ED	9, 12, 14, 18, 20
	Edward work area, Ontario	6	EE, EF	8, 12, 19, 20
	Hugh work area, Ontario	4	EG, EH	6, 9, 12, 15,
	Sauble, Ontario	5	EI	11, 12, 14, 16
	Sandbanks, Ontario	5	EJ	9, 10, 12, 20
	Midhurst, Ontario	5	EK	12, 17
	Tottenham / Orangeville / Hendrie, Ontario	5	EL	9, 12, 13, 20
Portugal	Moncao	2	P	NA

Table 2. Diversity estimates of *I. leucospoides* for different locations, from the COI and ITS sequence data. The first value given is calculated from the COI data and the second value from the ITS data.

Locality	N	NH	H	S	Pi
Australia	40 / 34	3 / 3	0.512 / 0.599	5 / 4	0.00298 / 0.00152
S. Africa	36 / 19	3 / 2	0.375 / 0.515	8 / 1	0.00322 / 0.00068
S. America	16 / 11	1 / 2	0 / 0.509	0 / 1	0 / 0.00067
N. America	41 / 37	15 / 1	0.855 / 0	14 / 0	0.00360 / 0

Note: There were only two sequences from Portugal which were identical, so these data were not included in the table. N = sample size; NH = number of haplotypes; H = haplotype diversity; S = number of polymorphic sites; Pi = nucleotide diversity

Table 3. Sequence divergence (%) between CO1 haplotype groups.

	Group 1	Group 2	Group 3	Group 4
Group 1	-	0.2	1.1	1.6
Group 2		-	1.5	1.9
Group 3			-	2.8
Group 4				-

Figure 1. Haplotype network showing the relationship between the 20 mitochondrial haplotypes of *I. leucospoides*. The colour of the circle indicates the geographic region where that haplotype is present and the size of the circle indicates the number of samples in each haplotype, as indicated by the scale provided. Solid black circles indicate hypothesized intermediate haplotypes. Lines between haplotypes indicate a one step mutational change. The haplotype number is indicated inside the circles. The haplotype numbers correlate with those in Table 1 and the haplotype groups with those in Fig. 2.

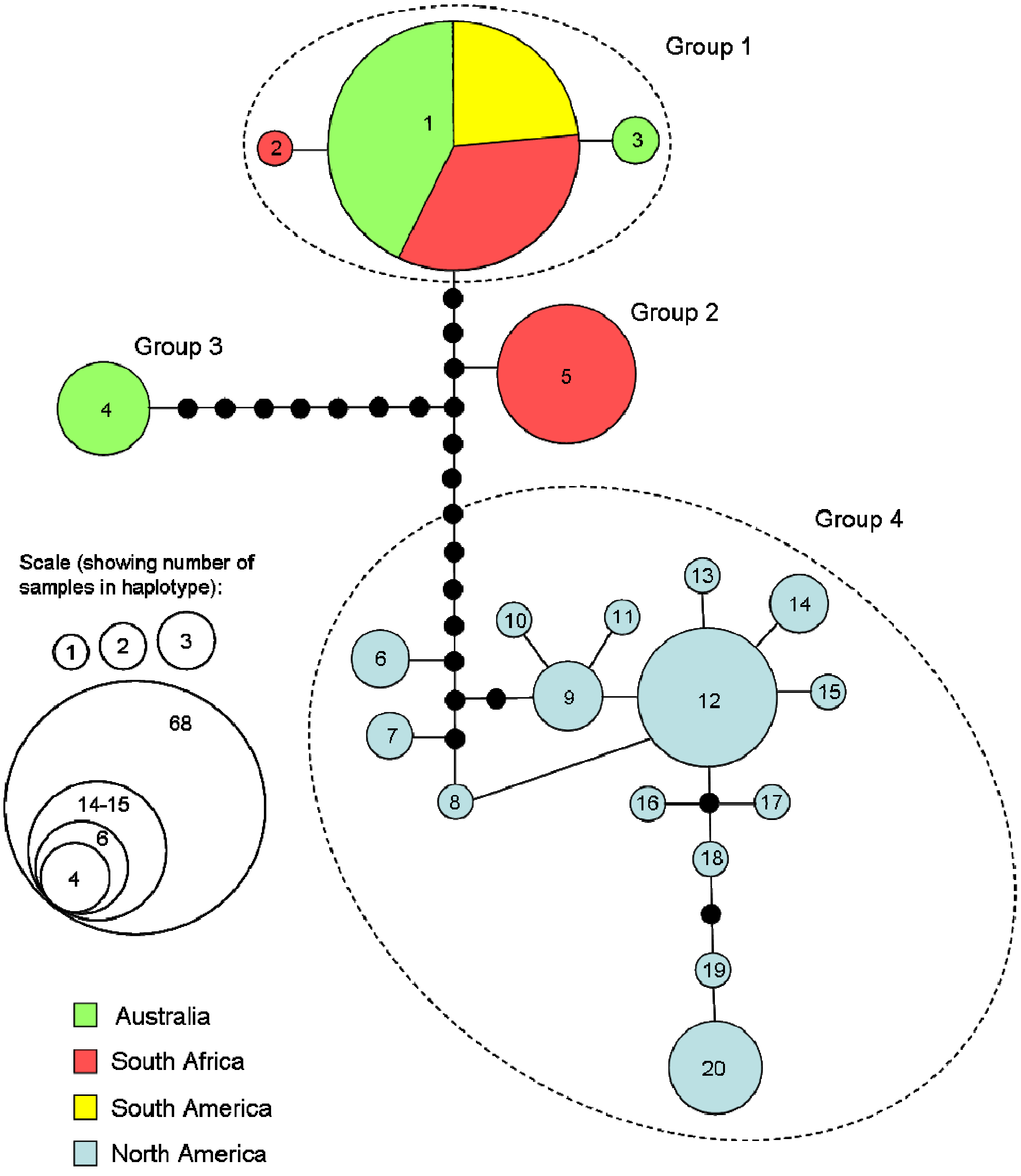
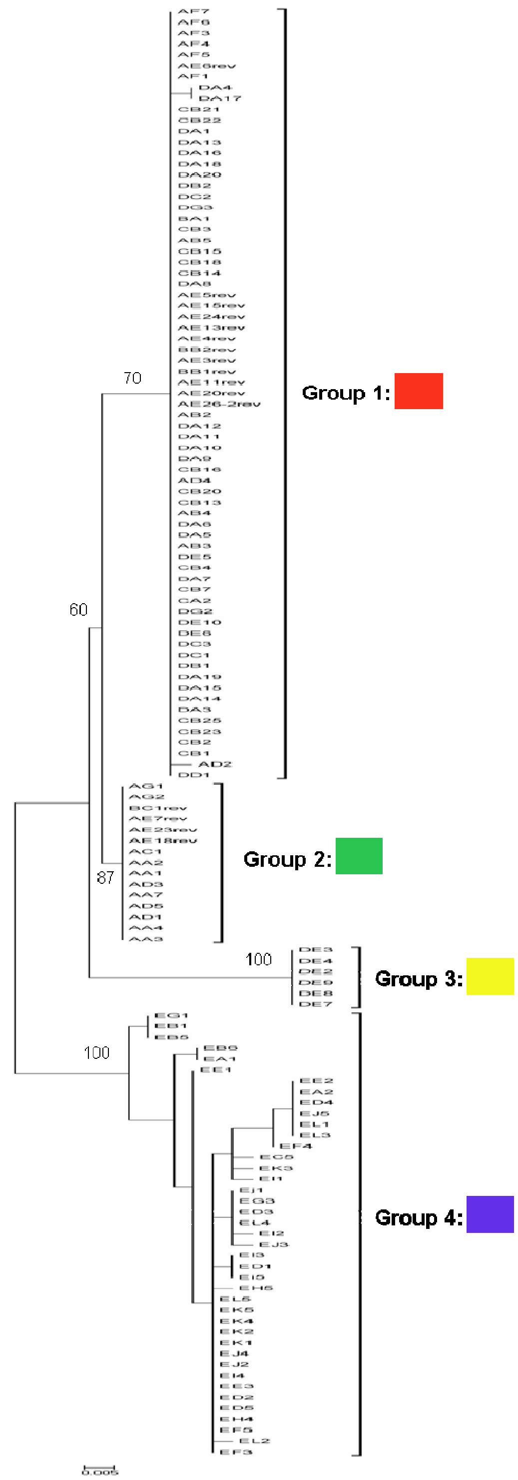
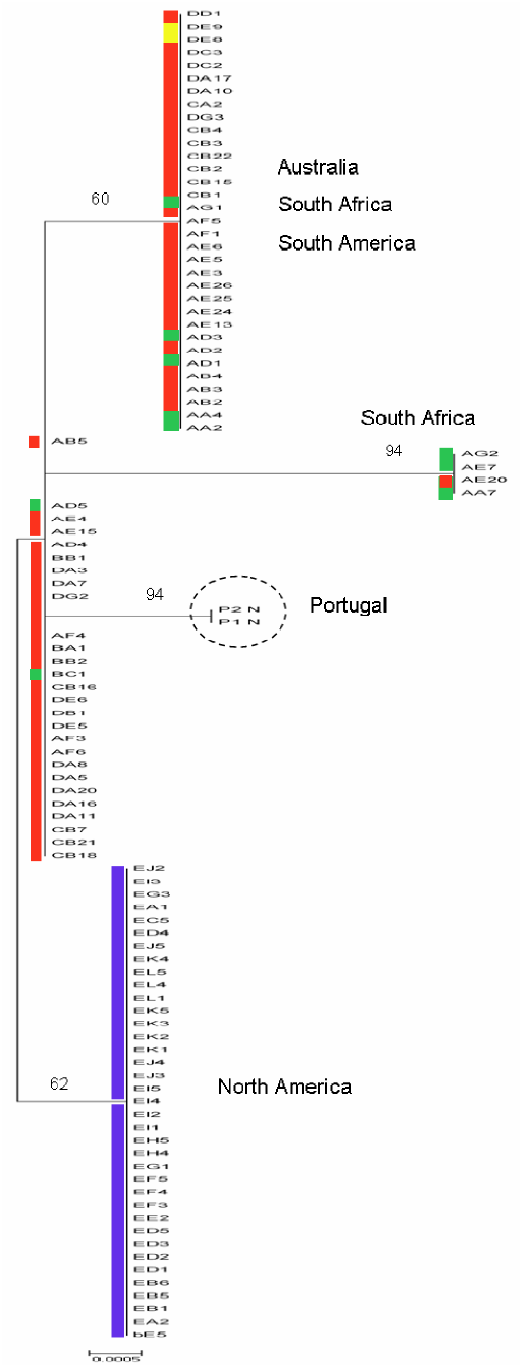

Group 1
1
2
3
Group 2
5
Group 3
4
Group 4
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Scale (showing number of samples in haplotype):
1
2
3
68
14-15
6
4
🟩 Australia
🟥 South Africa
🟨 South America
▨ North America

Figure 2. Maximum likelihood trees for the COI gene region (left) and ITS gene region (right). Mitochondrial haplotype groups from Fig. 1 are shown on both trees. Sample names correlate to those in Table 1. Bootstrap values for major branches are indicated at the nodes.


70
60
87
100
100
Group 1: 🟥
Group 2: 🟩
Group 3: 🟨
Group 4: 🟪
0.005


60
Australia
South Africa
South America
South Africa
94
94
Portugal
62
North America
0.0005

CHAPTER FIVE

Perception and knowledge of the Sirex woodwasp, *Sirex noctilio*, and other forest pest threats in the South African forestry community

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Sirex noctilio is one of the most serious invasive pests of pine. In South Africa, there has been a national effort to control *S. noctilio*, which has included increasing awareness of the pest amongst the local forestry community. In this study, we considered the impacts that the arrival of the pest and the awareness campaign has had on perceptions and knowledge of *S. noctilio,* as well as other forestry pests, amongst members of the forestry community. For the data collection, a survey questionnaire was developed and used in telephonic interviews. Results of the study showed that the Sirex awareness campaign had increased awareness of forestry pests in general. However, basic knowledge regarding the identification and symptoms of specific pests, such as *S. noctilio*, was poor. This will negatively influence monitoring efficacy. Traditional paper-based media and personal contact contributed most to enhanced awareness. Electronic media were less effective and improvement would require a more focused effort. It was of concern that private farmers and contractors, as well as non-English and non-Afrikaans speakers, were less well informed about forestry pests. Clearly, a fragmented landscape in terms of ownership and language, presents challenges for effective communication of forestry pest threats in South Africa.

Introduction

Pests and pathogens pose a serious threat to forestry worldwide. This threat includes both environmental disturbance by altering natural forest habitat, and economic losses, including direct losses to the crop and indirect losses due to the required management interventions (FAO 2009, Holmes *et al.* 2009). The increase in global trade and travel has added to this threat by the consequential increase in invasive pests and pathogens (Haack 2001, Wingfield *et al.* 2008). These invasions are particularly threatening when pests and pathogens from the native range of the host tree are introduced to environments where these trees are planted as non-natives in plantations. In such cases, the presence of a host monoculture for the pest or pathogen and a lack of their natural enemies provide favourable conditions for their rapid expansion.

In South Africa, a small natural forest resource led to the introduction of non-native tree species in the eighteenth and nineteenth centuries. Species of *Pinus*, *Eucalyptus* and *Acacia* were thus used to establish plantations to service the demand for wood and wood products (Owen and van der Zel 2000). This led to a forestry industry based on non-native trees that occupies about 1 % of the land surface (about 1.3 million ha), produces nearly 18 million tons of roundwood per annum and is a major contributor to the South African economy (Godsmark 2008). Subsequent to its establishment, the South African forestry industry has been increasingly plagued by invasive pests and pathogens (Wingfield *et al.* 2008). Of these, the most serious invasive insect of pine has been the Sirex woodwasp, *Sirex noctilio* F. (Siricidae: Hymenoptera).

Sirex noctilio is native to Eurasia (Spradbery and Kirk 1978), but has been accidentally introduced to the southern hemisphere, where it has become a serious pest of pine species (Hurley *et al.* 2007), and more recently it was accidentally introduced in to the USA and Canada (de Groot *et al.* 2007, Hoebeke *et al.* 2005). Subsequent to its detection in South Africa in 1994, *S. noctilio* spread through three of the main forestry growing provinces. Together with its fungal symbiont *Amylostereum areolatum*, *S. noctilio* has caused extensive losses to pine, mainly in highly stocked *Pinus patula* stands planted for pulpwood (Hurley *et al.* 2007).

In 2002, due to increasing infestations of *S. noctilio* in South Africa, a national committee was established and tasked with identifying and implementing appropriate control strategies for *S. noctilio*. This committee included representatives from government, private companies and landowners, and research organizations. An important component of the committee's strategy was to increase the awareness of *S. noctilio* within the forestry community. This was viewed as crucially important to ensure early detection of the pest and to obtain support for the management operations, such as the introduction of biological control agents. Efforts to promote the awareness of *S. noctilio* included information presented at conferences and field days, various forms of paper-based media, and information on the internet. However, the impact that these initiatives have had on the knowledge and perception of *S. noctilio* (and other forestry pests) amongst members of the forestry community is unknown.

Questionnaire-based surveys represent a tool that is commonly used to assess the perception and knowledge of invasive plants (Andreu *et al.* 2009, Bardsley and Edwards-Jones 2006, García-Llorente *et al.* 2008), agricultural or agroforestry insect pests (Nyeko *et al.* 2002, Obipile *et al.* 2008, Rubia *et al.* 1996), insect pests of native and exotic forestry (Chang *et al.* 2009, Flint 2006, McFarlane *et al.* 2006, Nyeko *et al.* 2007) and other invasive species (Bremner and Park 2007). Various methods can be used to collect information for a survey, including personal interviews, posted or e-mailed questionnaires, web-accessed questionnaires and telephonic interviews. Telephonic interviews offer some advantages, in that the study can be completed over a short period, substantial information can be collected within 15 to 30 minutes, there is effective sample control, ambiguities in the responses can be resolved and they have a high response rate (Churchill and Lacobucci 2002, Proctor 2005).

In this study we conducted a questionnaire using telephonic interviews to examine the current perception and knowledge of *S. noctilio* and other forest pests, amongst members of the South African forestry community. We furthermore considered how these perceptions were influenced by the socio-demographic characteristics of the respondents. Perception in this study was defined as "a way of understanding or regarding something", and knowledge as "information or skills gained through experience or education; awareness gained by experience of a fact or situation" (Oxford Dictionary of Current English 2001). We considered four key hypotheses, including: (1) whether knowledge of *S. noctilio* would be positively correlated with general pest knowledge; (2) whether perception of pests in general would influence knowledge of these pests; (3) whether exposure to elements of the *S. noctilio* awareness campaign would increase knowledge of *S. noctilio*; (4) whether socio-demographic characteristics of the respondents, such as job position, work experience, location, mother tongue and importance of major tree genera would influence the knowledge and perception of *S. noctilio* and forestry pests in general, and the exposure of the respondents to different *S. noctilio* awareness media.

Methods

Survey methods and questionnaire design

The target population for the survey was those people involved with forestry in South Africa, and who by the nature of their work should be aware of forestry pests. This included private timber growers, contractors, plantation management, researchers and senior management. Administrative staff, nursery staff and those working in paper, pulp and saw-timber mills were excluded. The sampling framework for the target population was a list of the relevant employees supplied by companies, government and a co-operative that deals with private farmers. In addition, relevant people in the existing lists of contacts of the Institute for Commercial Forestry Research (ICFR) and the Forestry and Agricultural Biotechnology Institute (FABI) were included. The complete list of people who could be contacted was not entirely comprehensive, but provided sufficient numbers for the sample required.

After the contact list had been assembled, it was refined to exclude invalid names and duplicates. The final list amounted to 561 contacts. These contacts were from the main forestry provinces in South Africa, including the Western Cape, Eastern Cape, KwaZulu-Natal, Mpumalanga and Northern Province (Fig. 1). The desired sample size was a minimum of 232. Sampling from the list of available contacts was random, but a minimum number of respondents was set for the provinces, based on the proportion of the given contacts living in that province and the importance of the province to forestry.

A questionnaire was prepared to assess the knowledge and perception regarding *S. noctilio* as well as forestry pests in general (Table 1). The questionnaire comprised six main sections: (1) questions to obtain socio-demographic information, namely job position, work experience, location, mother tongue, importance of major forestry species and presence of *S. noctilio* in the working environment; (2) questions pertaining to general pest perception regarding importance of the impact, research and monitoring of forestry pests; (3) questions pertaining to the knowledge of some of the major forestry pests (scientific and common names were given); (4) questions pertaining to the exposure of the respondent to different media channels that had been used to increase the awareness of *S. noctilio*; (5) questions pertaining to knowledge of *S. noctilio*; and (6) questions pertaining to the perception of *S. noctilio* regarding its control, impact and time required to manage the pest. The questions were preceded by a short introduction that explained the nature and importance of the survey.

The questions were prepared with the following objectives in mind: (1) translate information required into specific, unambiguous and answerable questions; (2) motivate cooperation from respondents; (3) minimize response errors (inaccurate answers); (4) only collect relevant information (Malhotra 2004, Zikmund 2000). The questionnaire consisted of closed questions that were either dichotomous, categorical, multiple choice or scale questions (Table 1). An exception was for the questions that called for the number of years work experience, where the actual number of years was given. Scale questions were from 1-10, where a response of one indicated the lowest and 10 the highest level of importance. Where appropriate, an option for “Don’t know” was given. The last question in the section dealing with knowledge of general forestry pests asked whether the respondent was aware of or had heard of *S. noctilio*. Where the response was negative, the questionnaire was terminated as the respondent would not be able to answer questions regarding their knowledge and perception of *S. noctilio*. However, the information collected concerning their knowledge and perception of forestry pests in general was still used. Thus, this question served as a qualifier to complete the remainder of the interview.

The questionnaire survey was conducted by means of telephonic interviews provided by Consulta Research (Pretoria, South Africa). Because a pre-testing of any questionnaire is vital (Churchill and Lacobucci 2002), this was done on five individuals who were not part of the target population. The pre-testing stage made it possible to determine the clarity of the questions, particularly concerning the pronunciation of scientific names and the length of the interview, and it ensured that the respondents had a clear understanding of the questions. The interviews were carried out from 15-26 June 2009.

Data editing and analysis

The data arising from the interviews was edited for errors and incomplete interviews were discarded. Where the respondent answered 'other' for job position, the response was changed to one of the existing categories where appropriate, or the interview was discarded (for example where the respondent indicated that he / she belonged to the nursery staff or administration). Interviews that contained missing fields were not considered. This excluded the interviews that terminated due to the qualifier question. Two variables were derived from the data set. The first derived variable indicated the number of general forestry pests known by the respondent (score out of five; considered as a categorical variable) and the other derived variable indicated the number of correct answers the respondent gave to the questions pertaining to their knowledge of *S. noctilio* (score out of 17; considered as a continuous variable). These derived variables were used in subsequent analyses to represent knowledge for general forestry pests and knowledge of *S. noctilio*, respectively.

Statistical analyses were performed using SAS ver. 8.2 (SAS 2001). An analysis of variance (ANOVA) using a general linear model (GLM) procedure was used to examine factors that influence the knowledge of *S. noctilio*, specifically socio-demographic characteristics and exposure to different awareness media, where the derived variable (knowledge of *S. noctilio*) was continuous. Logistic regression was used to examine influencing factors where the derived variable was not continuous. This included an examination of how socio-demographic characteristics influenced the response to questions pertaining to the perception of *S. noctilio*, exposure to awareness media and knowledge and perception of general forestry pests. A Kendall Tau correlation was used to examine the correlation between knowledge of *S. noctilio* and knowledge and perception of general forestry pests. Descriptive statistics (including mean, standard deviation, standard error and 95 % confidence levels) were calculated to show the perception of general forestry pests and *S. noctilio*.

Variables were re-categorized for their use in the logistic regression analysis. This was based on an examination of frequency tables, where categories with low frequencies were grouped together. For the job description variable, contractors and private farmers were grouped together and those who responded as ‘other’ were categorized into an existing group, where appropriate, or they were discarded. Work experience was categorized as five years or less, more than five years but less than 20 years, and 20 years or more. Location was defined based on provinces where *S. noctilio* had been detected and provinces where *S. noctilio* had not been detected. For mother tongue, all languages besides English and Afrikaans were grouped together. All scale questions (1-10) were changed to a binary response where a score of 1-7 was considered as not very important and a score of 8-10 was considered as very important. For the question “How long will it take to manage Sirex below economically important levels”, the responses were placed in two categories representing five years or less and more than five years, the later of which included the ‘never’ responses. Where the respondents provided a ‘Don’t know’ response, for this as well as other questions, these responses were not included in the analyses.

Results

Socio-demographic characteristics

A total of 240 completed interviews were obtained, which gave an error level of 4.8 % at a confidence level of 95 %. In terms of demographic characteristics of the respondents, over half (53 %) of the respondents were involved in plantation management, which included foresters, district foresters and area managers (Table 2). The remaining respondents were divided nearly equally among senior management, researchers, and private farmers and contractors. Most of the respondents had been involved in forestry for more than five years. Just over half (55 %) of the respondents were located in a province where *S. noctilio* was present, but only 43 % had *S. noctilio* in the plantation, farm or area in which they worked or for which they had responsibility. This was most likely because some of the respondents residing in areas where *S. noctilio* was present were not involved in pine forestry. Interestingly, the majority of respondents indicated their mother tongue as Afrikaans (Germanic language most closely related to Flemish and Dutch, spoken in South Africa and Namibia). In total, nine of South Africa’s 11 official languages were represented as mother tongue languages of the respondents, with only isiNdebele and Setswana not represented. Other languages included Shona (Zimbabwe and southern Mozambique), Luganda (Uganda) and German. The majority of respondents considered pine and eucalypts as very important, and wattle as not very important to their work.

Knowledge and perception of general forestry pests

There was a high knowledge of general forestry pests. In this case, 94 % of the respondents knew of at least three of the main forestry pests mentioned, 75 % knew at least four and 45 % knew all of them. *Sirex noctilio* and *Gonipterus scutellatus* were the pests that were best known by the respondents, with 98 % and 93 % respectively of the respondents being aware of or having heard of them. Only four of the respondents (2 %) had never heard of *S. noctilio* and they were, therefore, unable to continue with the questions pertaining to their knowledge and perception of *S. noctilio*. Whitegrubs, *Leptocybe invasa* and *Thaumastocoris peregrinus* were not as well known as *S. noctilio* and *G. scutellatus*, with 77 %, 74 % and 70 % respectively, of the respondents being aware of or having heard of them. How important the respondents considered eucalypts for their work significantly influenced their knowledge of general forestry pests (p = 0.02). Thus, those respondents who considered eucalypts to be very important in their work were more likely to have a greater knowledge of general forest pests. None of the other socio-demographic characteristics significantly influenced the knowledge of general forestry pests (Table 3).

The majority of respondents considered the impact of forestry pests on plantation forestry in South Africa to be very important (mean score = 8.3, SD = 1.7). Research on forestry pests and monitoring for forestry pests were perceived to be very important for their control (mean score = 9.0, 9.0, SD = 1.3, 1.4, respectively). The level to which respondents considered pine and eucalypts important to their work significantly influenced their perception of the importance of the impact of forestry pests (Table 3). Here, those that considered pine and eucalypts to be very important to their work were more likely to consider the impact of forestry pests as very important.

The mother tongue of the respondent significantly influenced their perception on the importance of research on forestry pests (Table 3). Those whose mother tongue was Afrikaans or English were more likely to consider research on forestry pests as very important. None of the other socio-demographic characteristics significantly influenced the perception of general forestry pests. Those who perceived research on forestry pests to be very important were more likely to have a greater knowledge of general forestry pests than those that did not consider research on forestry pests as very important (p = 0.04).

Sirex awareness media

Results showed that most of the media avenues used to increase awareness of *S. noctilio* had provided a high degree of exposure (Fig. 2). All the different media tools, except conferences and the internet / websites, had reached over 80 % of the respondents. Only 67 % of the respondents had heard of *S. noctilio* at a conference and only 51 % of the respondents were informed of *S. noctilio* through the internet / website. Respondents also indicated they had been exposed to *S. noctilio* via emails, ‘word of mouth’, personal communication with staff of the ICFR and TPCP (Tree

Protection Cooperative Programme), internal meetings / communications, studies at tertiary institutions, or that they had seen *S. noctilio* themselves.

Certain socio-demographic characteristics significantly influenced whether respondents had been exposed to some of the awareness media (Table 3). Job position significantly influenced whether the respondent was exposed to *S. noctilio* through posters, pamphlets or pests identification cards and conferences. Work experience significantly influenced whether a respondent had been exposed to knowledge of *S. noctilio* through pamphlets or pest identification cards. Furthermore, geographic location of the respondents significantly influenced whether they had been exposed to *S. noctilio* through magazines or newspapers and field days. Those involved in plantation management were more likely to have been made aware of *S. noctilio* through posters, followed by senior management, researchers and private farmers and contractors. Private farmers and contractors, and those with the least work experience were the least likely to have been exposed to *S. noctilio* through pamphlets or pest identification cards. Researchers were the most likely to have been exposed to *S. noctilio* at conferences and by other types of media (listed above), followed by senior management, plantation management, and private farmers and contractors. Those living in a province where *S. noctilio* was present were more likely to have been exposed to knowledge of *S. noctilio* through magazines and newspapers or at field days. The mother tongue of the respondent did not significantly influence their exposure to any of the media (Table 3).

Knowledge and perception of *S. noctilio*

Of the 17 questions asked to assess the knowledge of *S. noctilio*, the average number of correct answers from the respondent was 12.1 (71 %) (SD = 3.1; range = 3-17). Most of the respondents had a basic knowledge of the trees infested by *S. noctilio* (pine) and the age of trees infested (generally above seven years old), but knowledge regarding the identification of *S. noctilio* and symptoms of infestation were poor (below 50 % of the respondents) (Fig. 3). Regarding the distribution of *S. noctilio*, most respondents answered correctly that *S. noctilio* had been detected in the Western Cape, Eastern Cape and KwaZulu-Natal and not yet reached the Northern Province. However, only 40 % of the respondents answered correctly that *S. noctilio* had not been detected in Mpumalanga at the time that the survey was conducted (Fig. 3). Most of the respondents knew that *S. noctilio* could spread by natural flight, round wood, and on wooden pallets, but only 60 % of respondents knew that *S. noctilio* could not spread via the movement of bark (Fig. 3). Most of the respondents were aware that parasitic nematodes and plantation management were being used to control *S. noctilio* in South Africa, but less (65 %) knew that parasitic wasps are also used (Fig. 3).

Knowledge of *S. noctilio* was significantly influenced by the job position and mother tongue of the respondent (Table 4). Private farmers and contractors and those whose mother tongue was not Afrikaans or English had significantly less knowledge of *S. noctilio*. Exposure to *S. noctilio* awareness media significantly influenced knowledge of *S. noctilio* for all the media except articles in ICFR / TPCP newsletters and internet / website, where those exposed to these media had a greater knowledge of *S. noctilio* than those who were not (Table 4). There was a significant positive correlation, although weak, between the knowledge of *S. noctilio* and knowledge of general forestry pests ($r_k = 0.32$, $p < 0.0001$) (Table 5). There was no correlation between knowledge of *S. noctilio* and the perception of general forestry pests, relating to the importance of their impact, importance of research and importance of monitoring (Table 5).

Perception regarding the importance of using parasitic nematodes, parasitic wasps and plantation management to control *S. noctilio* was very high (score of above 8 on a scale of 1-10) (Fig. 4). The use of parasitic nematodes was, on average, perceived to be most important and the use of parasitic wasps the least important, but these differences were not significant. *Sirex noctilio* was perceived as a very serious problem for both current and future forest productivity in South Africa (score of above 8 on a scale of 1-10) (Fig. 4). The perceived threat to future productivity was slightly higher, but this difference was not significant. The majority of the respondents thought that *S. noctilio* would be managed below economically important levels within the next five years (42 %) or in more than five years (48 %). Only 3 % thought that *S. noctilio* would be managed below economically important levels within the next year and only 2 % thought that *S. noctilio* would never be managed below economically important levels. The remaining 5 % did not know when *S. noctilio* would be managed below economically important levels.

The perception of *S. noctilio* was influenced by certain socio-demographic characteristics of the respondents (Table 3). Job position significantly influenced the perception of how important plantation management was to control *S. noctilio*, the threat of *S. noctilio* to future forest productivity and the time needed to control *S. noctilio* below economically important levels. The perceived importance of plantation management was highest for plantation managers, then for researchers, private farmers and contractors, and lowest for senior management. Plantation managers were more likely to consider the threat of *S. noctilio* to future forest productivity as very high, and this level of importance was perceived as progressively lower by private farmers and contractors, researchers and senior management. Researchers were most likely to consider that *S. noctilio* would be controlled below economically important levels in five years or less (68 % of researchers), followed by senior management (50 %), plantation management (46 %) and private farmers and contractors (28 %). The importance of pine in their work significantly influenced the perception of the threat of *S. noctilio* to current and future forest productivity. Those respondents that considered pine very important in their work were more likely to consider the threat of *S. noctilio* to current and future forest productivity as very high. The importance of eucalypts in their work also significantly influenced the perception of the threat of *S. noctilio* to future forest productivity. Thus, those respondents that considered eucalypts very important in their work were more likely to consider the threat of *S. noctilio* to future forest productivity as very important.

Discussion

This study examined the current perception and awareness (knowledge) of *S. noctilio* and general forest pests in South African forestry, and how these are influenced by various socio-demographic factors relevant to this community. The results showed that: (1) knowledge of *S. noctilio* was positively correlated with knowledge of pests in general; (2) perception regarding pests in general did influence the knowledge of these pests; (3) exposure to *S. noctilio* awareness media increased knowledge of *S. noctilio*; (4) socio-demographic characteristics influence the knowledge and perception of *S. noctilio* and forestry pests in general, as well as the exposure to *S. noctilio* awareness media. A closer examination of the results revealed various successes and failures in efforts to increase awareness of *S. noctilio* in the forestry community. They also provided useful information on how a serious pest threat and efforts to control it can influence the knowledge and perception amongst members of the affected community.

Knowledge of general forestry pests and *S. noctilio*

Awareness of the most important forestry pests was relatively high, but it should be more acute, considering that the pests listed can cause serious losses. Besides *S. noctilio*, *G. scutellatus* was the pest that most respondents were aware of. *Gonipterus scutellatus* is an invasive pest of *Eucalyptus*, native to Australia, which was first detected in South Africa in 1916 (Tooke 1955). Its presence as a serious pest of various *Eucalyptus* species for nearly 100 years in South Africa explains why this insect is very well known. *Thaumastocoris peregrinus* and *L. invasa* are also serious invasive pests of *Eucalyptus* spp., but their appearance in South Africa has been much more recent, which probably explains why these pests are not as well known as *G. scutellatus*. *Thaumastocoris peregrinus* was first detected in South Africa in 2003, and on plantation *Eucalyptus* in 2005 (Jacobs and Nesser 2005, Nadel *et al.* 2009), and *L. invasa* was first detected in 2007 (Dittrich-Shröder *et al.* 2008), and on plantation *Eucalyptus* in 2009. Whitegrubs are larvae of native Scarabeiidae beetles and are pests of various plants, including forestry species (Govender 2007). Insecticides can be used to control whitegrubs which has possibly reduced the pest status and awareness of these insects.

Almost all respondents were aware of *S. noctilio*. This included people from different geographic locations, work experience, home language and differing importance of pine in their work environment. This can most likely be attributed to the major economic threat that *S. noctilio* poses for forestry in South Africa (Hurley *et al.* 2007). Consequently, the publicity that it has received, in media such as those considered in this study, including posters and pamphlets, field days, as well as word of mouth and other media, has been a vehicle to disseminate information.

Although awareness of *S. noctilio* was very high, it is of concern that there was a low basic knowledge regarding characteristics needed to identify the insect and symptoms associated with its damage. It is important that those involved in forestry are able to identify *S. noctilio* and its symptoms in order to ensure early detection of *S. noctilio* in new areas of infestation. This will ensure that management strategies can be established when the population of the insect is still low (Haugen *et al.* 1990). Clearly, future efforts to disseminate information regarding *S. noctilio* will need to focus on identification of the insect and symptoms associated with infestation.

Parasitic wasps have been used to a lesser extent than parasitic nematodes to manage *S. noctilio* populations. This must explain why control based on the use of these parasitoids was less well known. The confusion surrounding the current distribution of *S. noctilio* was to be expected as *S. noctilio* had been detected on the border of Mpumalanga at the time of the survey. This explains why many of the respondents thought that *S. noctilio* was already in that Province. A lack of accurate knowledge regarding the distribution of the insect can, however, affect the spread of the insect if people move infested wood because they perceive the insect to already be present in an area. This is a matter that will require attention in the future.

Perception of forestry pests and how this influences general pest knowledge

The impact that forestry pests have on plantation productivity, as well as the importance of research and monitoring aimed at their control was perceived as very important. This reflects the seriousness of pest infestations in South African plantation forestry. *Sirex noctilio* alone was estimated to result in approximately R300 million losses per annum to the forestry industry during its peak in the KwaZulu-Natal province (Hurley *et al.* 2007). Infestations of *G. scutellatus* are increasing. *Thaumastocoris peregrinus* has spread rapidly through South Africa and is now considered one of the main pests of *Eucalyptus* (Nadel *et al.* 2009). Likewise, *L. invasa*, although only recently detected in plantations, poses a serious threat to *Eucalyptus* forestry (Dittrich-Shröder *et al.* 2008). Three of these invasive pests have been detected in the last 15 years and indications are that the rate of appearance of new invasive pests is increasing with time (Wingfield *et al.* 2008). The seriousness of native pest infestations is also increasing with the cossid moth, *Coryphodema tristis* causing extensive losses of *E. nitens* (Boreham 2006, Gebeyehu *et al.* 2005). Infestations of these pests are particularly serious as in most cases effective control measures are not available.

The perception that people have of pests can influence their knowledge of pests in general. Our study showed that those respondents that perceived research on forestry pests and their control to be very important had a better knowledge of forestry pests. However, this influence was only marginally significant and the perception of the impact of forestry pests and monitoring aimed at controlling them did not significantly influence general pest knowledge.

Exposure to *S. noctilio* awareness media

Awareness campaigns provide an effective means to support the management of pests and to share information regarding their impact (Garcia-Llorente *et al.* 2008). Heong *et al.* (1998, 2008) for example showed how different forms of communication, including radio broadcasts, information posters and leaflets, can be used effectively to alter management practices. The results of the present study indicate that the media used to increase awareness of *S. noctilio* has largely been effective in reaching members of the forestry community and significantly increased the knowledge of the pest. In particular, posters, pamphlets / pest identification cards, field days, and magazines and newspaper articles have had a high level of penetration into the forestry community and they have effectively increased knowledge of *S. noctilio*.

Conferences and the internet or websites were the least effective media to inform people about *S. noctilio*. Conferences are generally not attended by members of all sectors of the forestry community and only a limited number of people can attend these meetings due to restricted costs and time constraints. The poor reach of the internet or websites in promoting awareness of *S. noctilio* is possibly because many members of the forestry community do not have frequent access to the internet. Furthermore, at the time of the survey there was no website that provided a good source of information on *S. noctilio*. Although online advertising in South Africa recorded the fastest growth rate from all English-language countries in 2008, there is still a very slow growth in internet access, with only 8 % of the population estimated to have internet connection in 2007 (www.worldwideworx.com). An informative website on *S. noctilio* has recently been developed that is specifically targeted at the forestry community in South Africa. This is expected to increase knowledge of *S. noctilio* among those that have internet connections, but it will not assist those people without access to the internet.

A medium to promote knowledge of *S. noctilio* that could be very successful is the mobile phone. The use of mobile phones is increasing globally, including in Africa, and they are replacing land lines as the preferred means of communication (Hodge 2005). Most people in South Africa have a mobile phone (Esselaar and Stork 2005), and penetration using this medium will be high. Forms of communication will be much more limited, but potentially simple alerts with attached images could be dispatched, with the recipient directed to a contact number or website for more information should they suspect the presence of the pest in their area. This could be particularly useful in monitoring the spread of newly discovered pests.

Influence of socio-demographic characteristics

The influence of socio-demographic characteristics on the knowledge and perception of pests and other invasive species has been clearly shown in various studies (Chang *et al.* 2009, Garcia-Llorente *et al.* 2008, McFarlane *et al.* 2006, Nyeko *et al.* 2002, Obopile *et al.* 2008). The influence of socio-demographic characteristics on the exposure to awareness media is also important because these media make it possible to develop the knowledge and perception of a problem amongst members of a given community. This survey exposed the negative issue that private farmers and contractors were shown to have the least exposure to awareness media and the lowest knowledge of *S. noctilio*. This was not because of the importance of pine to these farmers and contractors, as this variable was not found to significantly influence exposure to awareness media or knowledge of *S. noctilio*. Rather, it appears that the media used to promote awareness regarding *S. noctilio* have not penetrated this sector of the forestry community as successfully as others, and this has resulted in the lower level of knowledge regarding *S. noctilio*.

Posters, pamphlets / pest identification cards and conferences were the particular media that failed to reach private farmers and contractors effectively. This weakness will need to be addressed in the future. The forestry sector in South Africa is currently undergoing significant, and government supported transformation (see http://www2.dwaf.gov.za/webapp/ProjectsBBBEE.aspx), which will continue to increase private ownership. The lack of awareness in this sector evident from this survey is consequently of particular concern.

Respondents of this survey whose mother tongue was neither English nor Afrikaans had a lower level of knowledge concerning *S. noctilio*. Furthermore, they considered the importance of research for the control of forestry pests as less important than those whose mother tongue was English or Afrikaans. The lower level of knowledge regarding *S. noctilio* was not due to the awareness media having been in English or Afrikaans, as mother tongue did not significantly influence exposure to awareness media. This result might at least partially be explained due to the higher percentage of private farmers and contractors that were included in this group of respondents (Table 6). Furthermore, the surveys were conducted in English with an option for Afrikaans, but not for the other languages spoken in South Africa, and this could have influenced how well the questions pertaining to the knowledge of *S. noctilio* were understood. The reason for why this group considered research for the control of forestry pests to be less important is unclear, and this difference was only marginally significant. The future transformation of ownership in the South African forestry industry will increase the number of non-English and non-Afrikaans speaking people owning plantations. Thus, home language will need to be more carefully considered in future awareness campaigns.

Four of the five pests mentioned in the general pest knowledge section of the questionnaire were pests of *Eucalyptus*. This explains why those respondents who considered *Eucalyptus* as very important in their work scored a higher knowledge of general forestry pests. These four pests and *S. noctilio*, which attack pine, have a serious impact on forest productivity. This then explains why the impact of forestry pests was perceived as higher by those who considered eucalypts and pine as very important in their work. The importance of wattle did not influence the perception of forestry pests in general. This was possibly because insect pests on wattle do not currently pose as serious a threat as do those on pine and eucalypts. Not surprisingly, those respondents that considered pine as very important also perceived the use of parasitic nematodes as biological control agents and the threat of *S. noctilio* as very important.

Interestingly, geographic location of respondents did not influence perception or knowledge of forestry pests in general or *S. noctilio*. It would have been expected that the perception and knowledge of *S. noctilio* would differ between areas where *S. noctilio* is present and those where the pest is absent, but this was not the case. This is in contrast to for instance the study of McFarlane *et al.* (2006) where public living in areas where the mountain pine beetle (*Dendroctonus ponderosae*) outbreaks occurred had a more informed view of the pest and they were also more likely to support efforts to control it. The lack of location affect on the knowledge and perception of *S. noctilio* is likely because it is a national threat to forestry and because efforts have been made to distribute information to all forestry areas.

Conclusions

The South African forestry community regards the threat of *S. noctilio* and other forestry pests, and the need to control them as extremely important. The national programme to increase the awareness of *S. noctilio* amongst members of the forestry community has largely been effective in increasing the knowledge of the pest. This will consequently increase chances of early detection of *S. noctilio* in new areas of infestation and increase the support for management operations. This result demonstrates the value of a nationally co-ordinated awareness campaign.

Where the awareness campaign failed was in its ability to reach private farmers and contractors. This demonstrates the affect that a fragmented and diverse forestry community poses for communication and, therefore, pest management. Pest management in the future will need to consider more effective means of communication in order to effectively reach all the sectors of the forestry community. The diversity of languages spoken amongst the target group also needs to be considered and there could be value in conducting marketing research to better understand this and other aspects of the target group.

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Table 1. Summary of survey questions

Questions	Scale / categories
Socio-demographic information	
Which one of the following describes your position the best?	6 categories[a]
How many years have you been involved in forestry?	Continuous
In which province do you work or are based in for the majority of time?	9 provinces
What is your mother tongue?	11 official languages
Indicate the importance of the following exotic tree species for your work: pine, eucalypts, wattle	Scale (1-10)[b]
Do you have Sirex in the area that you work in or are responsible for?	Yes / No
General pest perception	
How important do you consider the impact of forestry pests on plantation productivity in South Africa?	Scale (1-10)[b]
How important do you consider research on forestry pests for their control?	Scale (1-10)[b]
How important do you consider monitoring for the presence of forestry pests for their control?	Scale (1-10)[b]
General pest knowledge	
Which of the following forestry pests in South Africa are you aware of or have heard of: *Thaumastocoris peregrinus, Leptocybe invasa, Gonipterus scutellatus*, whitegrubs, *Sirex noctilio*? (common names were also given)	Yes / No
Exposure to awareness media	
Were you informed of Sirex through: posters; pamphlets or pest cards; articles in ICFR / TPCP newsletters; internet or website; magazines or newspapers; field days; conferences; other?	Yes / No[b]
Sirex knowledge	
(17 questions relating to knowledge on the identification, symptoms of attack, distribution and means of spread and control)	Multiple choice, Yes / No[b]
Sirex perception	
How important are the control measures: parasitic nematodes; parasitic wasps; plantation management?	Scale (1-10)[b]
How serious a problem is Sirex for forest productivity in South Africa (current and future)?	Scale (1-10)[b]
How long will it take to manage Sirex below economically important levels in South Africa?	5 categories[c]

[a] contractor, private farmer, plantation management, researcher, senior management, other

[b] respondants were also given the option "Don't know"

[c] within the next year; within the next five years; more than five years; never; don't know

Table 2. Socio-demographic characteristics of sample population

Socio-demographic characteristic	Category	% of sample population
Job position	Private farmers / contractors[a]	15
	Plantation management	53
	Researcher	14
	Senior management	18
Work experience[b]	Five or less years	15
	More than five years	47
	More than 20 years	38
Location	In Sirex-infested area[c]	55
	Not in Sirex-infested area[d]	45
Mother tongue	English	32
	Afrikaans	46
	Other[e]	22
Importance of pine	Very important[f]	79
	Not very important[g]	21
Importance of eucalypts	Very important[f]	70
	Not very important[g]	30
Importance of wattle	Very important[f]	21
	Not very important[g]	79
Presence of Sirex in working area	Yes	43
	No	57

[a] private farmers and contractors were combined for analyses

[b] years of work experience grouped into three categories for analyses

[c] Western Cape, Eastern Cape, KwaZulu-Natal

[d] Mpumalanga, Limpopo, Gauteng, Free State

[e] isiXhosa, isiZulu, Sepedi, Sesotho, Siswathi, Tshivenda, Xitsonga, Shona, Luganda, German

[f] score of 8-10

[g] score of 1-7

Table 3. Influence of socio-demographic characters on perception of *S. noctilio*, exposure to awareness media, general pest knowledge and general pest perception. Results are from logistic regression analysis, where the dependant variables were categorical.

	Socio-demographic characters							
	Job position	Work experience	Location	Home language	Importance of pine	Importance of eucalypts	Importance of wattle	Presence of Sirex woodwasp in working area
General pest knowledge	0.1	0.9	0.06	0.5	0.8	0.02*	0.1	0.8
General pest perception								
Impact of forestry pests	0.5	0.7	0.7	0.8	0.03*	0.03*	0.6	0.1
Research on forestry pests	0.6	0.2	0.4	0.04*	0.3	0.6	0.1	0.4
Monitoring of forestry pests	0.5	0.6	0.5	0.07	0.3	0.2	0.1	0.7
Exposure to awareness media								
Posters	0.003**	0.7	0.9	0.1	NA	NA	NA	NA
Pamphlets or pest cards	0.008**	0.0001***	0.8	0.1	NA	NA	NA	NA
Articles in TPCP / ICFR newsletters	0.1	0.3	0.8	0.1	NA	NA	NA	NA
Internet or website	0.2	0.4	0.9	0.9	NA	NA	NA	NA
Magazines or newspapers	0.2	0.1	0.03*	0.5	NA	NA	NA	NA
Field days	0.3	0.9	0.02*	0.3	NA	NA	NA	NA
Conferences	0.0002***	0.9	0.7	0.8	NA	NA	NA	NA
Other	0.05*	0.9	0.8	0.4	NA	NA	NA	NA
Sirex perception								
Importance of nematodes for control	0.4	0.4	0.4	0.9	0.02*	0.6	0.8	NA
Importance of wasps for control	0.3	0.6	0.7	0.5	0.8	0.1	0.2	NA
Importance of plantation management for control	0.01**	0.9	0.6	0.1	0.3	0.3	0.2	NA
Current threat of Sirex	0.2	0.9	0.08	0.3	0.004**	0.07	0.2	NA
Future threat of Sirex	0.05*	0.06	0.7	0.7	0.0004***	0.01**	0.2	NA
Time needed to control Sirex	0.03*	0.7	0.7	0.3	0.8	0.3	0.3	NA

*significant at 5% level, $p < 0.05$
**significant at 1% level, $p < 0.01$
***significant at 0.1% level, $p < 0.001$

Table 4. Factors affecting knowledge of *S. noctilio*. Results from ANOVA using general linear model, where dependant variable was continuous.

Variable	df	F value	p value
Socio-demographic characteristics			
Job position	3	10.03	<.0001***
Work experience	2	2.29	0.1
Location	1	0.98	0.32
Home language	2	8.45	0.0003***
Importance of pine	1	1.46	0.23
Importance of eucalypts	1	0	0.96
Importance of wattle	1	0.46	0.5
Presence of Sirex woodwasp in working area	1	1.48	0.23
***S. noctilio* awareness media**			
Posters	1	4.18	0.042*
Pamphlets or pest cards	1	4.9	0.028*
Articles in TPCP / ICFR newsletters	1	3.72	0.055
Internet or website	1	1.05	0.31
Magazines or newspapers	1	9.89	0.0019**
Field days	1	12.25	0.0006**
Conferences	1	10.87	0.0011**
Other	1	8.33	0.0043**

*significant at 5% level, $p < 0.05$
**significant at 1% level, $p < 0.01$
***significant at 0.1% level, $p < 0.001$

Table 5. Correlation between knowledge and perception of general forestry pests and knowledge of *S. noctilio.*

	r_k	p value	n
General pest knowledge	0.32	<0.0001	236
Importance of impact of forestry pests	-0.028	0.57	236
Importance of research on forestry pests	0.079	0.12	236
Importance of monitoring for presence of forestry pests	0.023	0.66	236

r_k = Kendall Tau Correlation Coeffecient

Table 6. Break-down of mother tongue groups by percentage of different job opportunities represented in each group

	Contractor / Private	Plantation managment	Researcher	Senior management	All
Afrikaans	10%	63%	8%	19%	100%
English	17%	36%	26%	21%	100%
Other	23%	60%	8%	9%	100%

Figure 1. Map of South Africa, showing provinces and pine plantation distribution (in black)


NORTHERN PROVINCE
NORTH WEST
GAUTENG
MPUMALANGA
SWAZILAND
NORTHERN CAPE
FREE STATE
KWAZULU/NATAL
LESOTHO
EASTERN CAPE
WESTERN CAPE
N
W
E
S

Figure 2. Exposure to different *S. noctilio* awareness media.

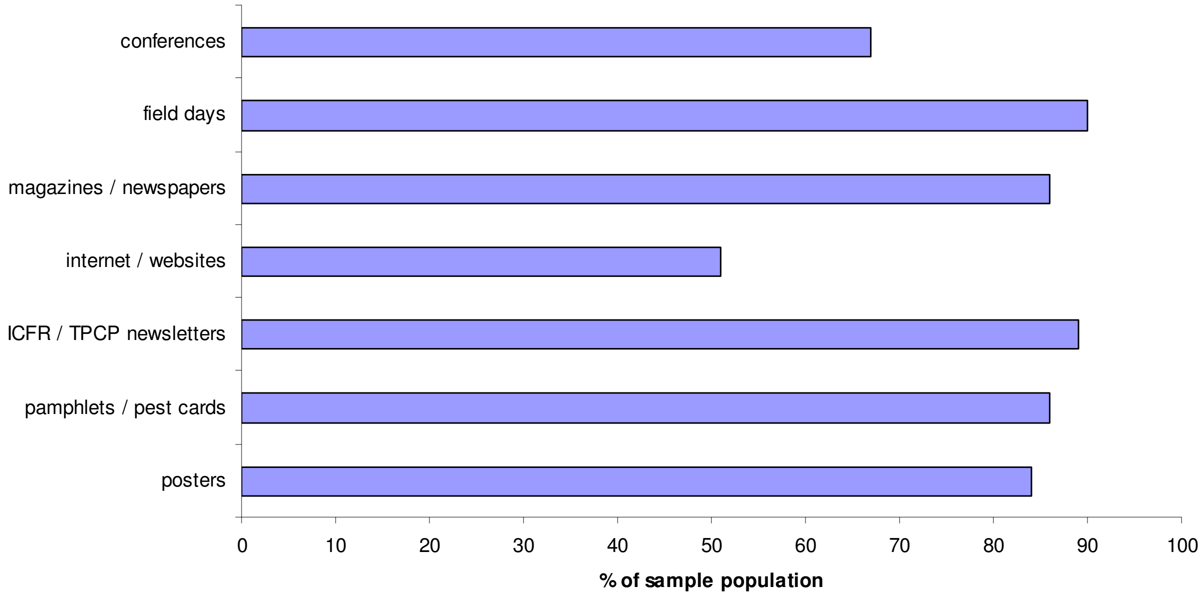

conferences
field days
magazines / newspapers
internet / websites
ICFR / TPCP newsletters
pamphlets / pest cards
posters
0
10
20
30
40
50
60
70
80
90
100
% of sample population

Figure 3. Knowledge of *S. noctilio*. Correct responses to questions around the identification, symptoms, distribution, and means of spread and control.

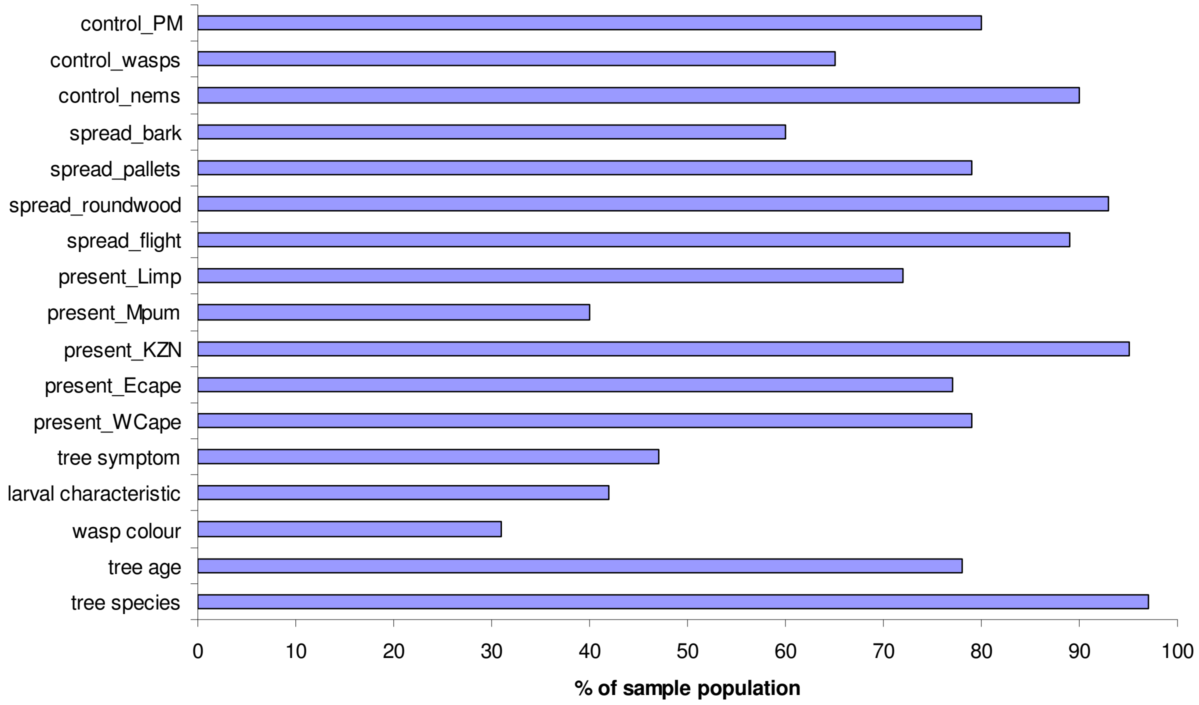
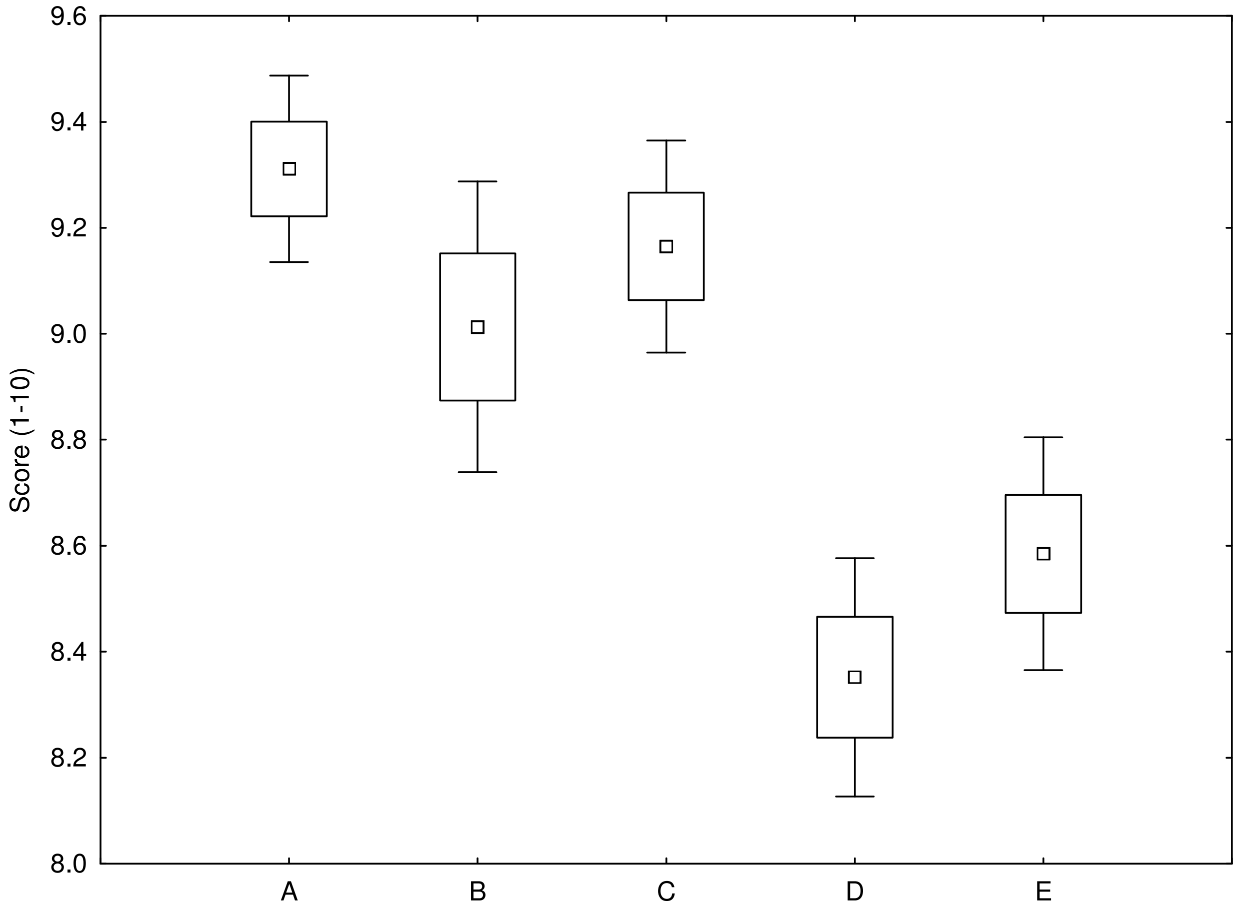

control_PM
control_wasps
control_nems
spread_bark
spread_pallets
spread_roundwood
spread_flight
present_Limp
present_Mpum
present_KZN
present_Ecape
present_WCape
tree symptom
larval characteristic
wasp colour
tree age
tree species
0
10
20
30
40
50
60
70
80
90
100
% of sample population

Figure 4. Perception of *S. noctilio*, as scored on a scale of 1-10, with one being not important and 10 being very important. A. Importance of nematodes to control *S. noctilio*. B. Importance of parasitic wasps to control *S. noctilio*. C. Importance of plantation management to control *S. noctilio*. D. Seriousness of *S. noctilio* for current forest productivity. E. Seriousness of *S. noctilio* for future forest productivity. Small squares show mean, large squares show standard error and error bars show 95 % confidence levels.


9.6
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
Score (1-10)
A
B
C
D
E

CHAPTER SIX

The control of the Sirex woodwasp in diverse environments: The South African experience

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The woodwasp *Sirex noctilio* was detected in South Africa in 1994, in Cape Town. Within the following 15 years, *S. noctilio* had spread across four of the five main forestry regions. Losses incurred from *S. noctilio* damage increased dramatically as it moved into the Eastern Cape and KwaZulu-Natal provinces, where densely stocked pulpwood stands provided ideal conditions for the population of the wasp to increase. Control strategies developed in Australia and New Zealand were adopted, including the introduction of the parasitic nematode *Deladenus siricidicola* and the parasitic wasp *Ibalia leucospoides*. Adaptations were required for the techniques used to release *D. siricidicola* due to the poor success obtained in the summer rainfall areas. These adaptations increased the effectiveness of the inoculations, although overall inoculation success remained poor. The inoculations spread the nematodes across infested areas; subsequent natural spread of the nematode has resulted in a steady increase in parasitism levels. *Ibalia leucospoides* was successfully released in the Western Cape in 1998, but only recently (since 2006) have there been efforts to release this parasitoid in other infested areas. Monitoring has been crucial for the early detection of infestations and release of biological control agents, and has included field surveys, lure-based traps and more recently trap trees. Plantation management strategies to combat *S. noctilio* have been used to some extent, but are restricted by economic considerations. Although infestations of *S. noctilio* have recently decreased, the movement of *S. noctilio* into the largest pine-growing province and the current inability to quickly establish biological control due to poor inoculation success, emphasizes the fact that *S. noctilio* continues to pose a serious threat to the pine industry in South Africa.

Introduction

The woodwasp *Sirex noctilio* (Siricidae: Hymenoptera) is a major pest in pine plantations throughout the southern hemisphere, where together with its fungal symbiont *Amylostereum areolatum*, it infests and kills *Pinus* spp. *Sirex noctilio* is native to Eurasia and North Africa and was first detected in the southern hemisphere around 1900 in New Zealand (Miller and Clarke 1935). During the next 100 years, *S. noctilio* spread across the southern hemisphere and is now present in Australasia, South America and South Africa (Carnegie *et al.* 2006, Hurley *et al.* 2007). Most recently, an established population of *S. noctilio* was recorded in the United States of America and Canada (Hoebeke *et al.* 2005, de Groot *et al.* 2007).

Due to the long history of *S. noctilio* in New Zealand (since 1900) and Australia (since 1952) (Gilbert and Miller 1952), and the extensive damage that it has caused in these countries (see Rawlings 1955 and Haugen 1990 as examples), management strategies to reduce the impact of *S. noctilio* were well established before the wasp spread to South America and South Africa. These management strategies were strongly focused on the establishment of biological control, although silvicultural measures such as thinning and timely pruning were also seen as important (Madden 1968, Neumann *et al.* 1987). Biological control of *S. noctilio*, which had been very successful in New Zealand and Australia (Hurley *et al.* 2007), was rapidly adopted in South America and South Africa when *S. noctilio* was first detected in these locations.

The control of *S. noctilio* in South America and South Africa has not been uniformly effective, specifically when compared to the success recorded in Australia and New Zealand (Hurley *et al.* 2007). In a detailed examination of *S. noctilio* infestations and their control in the southern hemisphere, Hurley *et al.* (2007) suggested that the integrated control approach for *S. noctilio* might require significant local adaptation in new areas of introduction for effective control to be obtained. The term local adaptation refers to the changes required to the adopted management program, in response to the difference in conditions that can influence the program's success between the local environment and the environment where the specific approach was developed. The aim of this chapter is to provide an overview of the efforts to control *S. noctilio* in South Africa, where the environment has dictated local adaptation of existing control strategies. Information on pine plantations and management, and the history of *S. noctilio* in South Africa is also provided.

Pine forestry in South Africa

South Africa has a low mean rainfall and limited natural forests. Savannah woodland covers 19 % of the land area, and closed canopy forest only 0.5 % (Owen and van der Zel 2000). The arrival and expansion of European colonies in South Africa, and the consequent demand for timber, rapidly placed a strain on the limited supply of wood resources. Thus, from the early 1700s attempts were made to protect natural forests and exotic tree species were introduced to supplement the demand for wood (Owen and van der Zel 2000). In the late seventeenth century, *P. sylvestris*, *P. pinea* and *P. pinaster* were introduced. Numerous pine species were introduced after this, including *P. radiata* (pre-1865), *P. taeda* (pre-1900), *P. patula* (1907) and *P. elliottii* (early 1900s) (Owen and van der Zel 2000). These are currently the four main pine species planted in South Africa (Poynton 1977). The first commercial plantations were established in 1875, where black wattle (*Acacia mearnsii*) was planted to provide railway sleepers, and later as a superior vegetable tanning material. By 1910, 60 000 ha were planted to *A. mearnsii*. Plantations of non-native tree species increased greatly after World War 2; these included species of *Pinus* and *Eucalyptus*. By 1960, 980 000 ha had been planted to non-native species and by 1975 the planted area had risen to 1.1 million ha (Owen and van der Zel 2000).

Plantation forests currently cover about 1.0 % of the land area (about 1.3 million ha) in South Africa, the majority of which is in the Mpumalanga (40.7 %) and KwaZulu-Natal (38.5 %) provinces. The forest industry produces nearly 18 million tons of roundwood per annum, and forest products exports contribute over R12 billion to foreign trade (third only to metal ores and coal), making forestry a major contributor to the South African economy (Godsmark 2008, based on 2007 figures). *Pinus* species make up about 53 % of the planted area, distributed in Mpumalanga (46.6 %), KwaZulu-Natal (21.5 %) and the Eastern and Western Cape (27.8 %). Most of the pine is used for saw timber (68.5 %), followed by pulpwood (30.4 %) and poles (0.9 %) (Godsmark 2008).

South African forests cover a broad range of climatic, soil and altitudinal zones (Figs 1-4). Site-species matching is thus a crucial aspect of South African forestry, although the presence and location of processing plants is also a major factor determining which pine species are planted. In the Western Cape, which is a winter rainfall and all-year rainfall area, *P. radiata* is most commonly planted. In KwaZulu-Natal, Mpumalanga and Northern Province, which are summer rainfall areas, *P. patula* is primarily planted, although *P. taeda* and *P. elliottii* are also widely utilised. *Pinus patula* is mainly planted at altitudes higher than 1000 m.a.s.l. (metres above sea level) due to its snow tolerance and its susceptibility to *Diplodia pinea* at lower altitudes.

Management strategies for *Pinus* spp. differ in the different geographical areas where plantations have been established. In the Western Cape, Eastern Cape and Northern Province, pine trees are predominantly grown for saw timber. In KwaZulu-Natal, pine trees are predominantly grown for pulpwood. In Mpumalanga, there are large areas of pine trees planted for both saw timber and pulpwood. Management for saw timber production includes pruning and thinning, where suppressed trees are removed. In contrast, management for pulpwood production generally does not include thinning, though pruning for access to a height of 2 m is performed, especially in *P. patula* stands. The absence of thinning in pulpwood production stands results in a much higher final stand density (approx. 800 - 1250 stems per hectare [spha]) than in saw timber production stands (approx. 250 spha), at the time of harvest.

Introduction and spread of *S. noctilio* in South Africa

Sirex noctilio was first detected in South Africa in 1962 where it was found in wood at a timber yard in Port Elizabeth (Taylor 1962). Infested material was destroyed and there were no further reports of the wasp in South Africa for the next three decades. In April 1994, *S. noctilio* was reported in *P. radiata* trees in Cape Town (Tribe 1995). It was estimated to have arrived at least two years prior to its detection. The introduction of *S. noctilio* into South Africa was likely from South America or its native range (Slippers *et al.* 2001).

During the next fifteen years, *S. noctilio* spread in a north-easterly direction, following the main pine resources in the country (Fig. 5). Surveys in the Cape region in 1994 detected *S. noctilio* as far as 90 km from Cape Town where it was first detected, infesting mainly *P. radiata* (Tribe 1995). By 1998, *S. noctilio* had been detected as far as Riversdale in the east and Van Rhynsdorp in the north (Tribe and Cillié 2004). In 2001, the wasp was found near Knysna (Brenton-on-Sea). In 2002, *S. noctilio* was detected in the Eastern Cape and KwaZulu-Natal (at Weza) in *P. patula* stands (Hurley *et al.* 2007). In these areas, the wasp rapidly reached epidemic proportions and gradually made its way northwards. By 2009, *S. noctilio* had spread throughout KwaZulu-Natal and was most recently recorded in Mpumalanga (November 2009) - South Africa's main pine growing province (authors, unpublished data). The spread of *S. noctilio* over the last fifteen years has likely been accelerated by accidental human-assisted transport, such as through the movement of infested logs and wood packaging material (Fig. 6A).

Although it was considered a major threat to pine forests in the Western Cape after its detection, *S. noctilio* has caused little damage in this province. The exception was an infestation in the George area where *S. noctilio* caused an average of 10 % mortality in an overstocked area of 12-13-year-old *P. radiata* (Hurley *et al.* 2007). In contrast, soon after the detection of *S. noctilio* in the Eastern Cape and KwaZulu-Natal, serious mortality occurred. The damage peaked in 2004-2006, where mortality in some stands was over 35 % and the mean mortality was 6 % (Hurley *et al.* 2007) (Fig. 6B).

Hurley *et al.* (2007) suggested that differences in management strategies between the provinces had contributed to differences in infestation levels. In contrast to the Eastern Cape and KwaZulu-Natal, the Western Cape does not grow trees for pulpwood and thus does not have large areas of unthinned, heavily stressed stands. In addition to the differences in management strategies, the slow establishment of biological control agents in the Eastern Cape and KwaZulu-Natal also contributed to the epidemic in this area. Infestation levels of *S. noctilio* have declined in KwaZulu-

Natal after 2006, and mean infestation levels in the area were below 1 % in 2009 (authors, unpublished data). Nonetheless, *S. noctilio* is still considered the most serious pest of pine currently in South Africa, based on resulting tree mortality and the threat it poses as it moves in to new pine areas.

Efforts to control *S. noctilio* in South Africa

Biological control: Parasitic nematodes

The nematode *Deladenus* (= *Beddingia*) *siricidicola* Bedding is considered the primary biological control agent for *S. noctilio* (Bedding and Iede 2005). This nematode has a complex life-cycle that includes fungus-feeding and parasitic phases (Bedding 1972). In the fungus-feeding form, *D. siricidicola* feeds on the *S. noctilio* fungal symbiont *A. areolatum*, and has a life-cycle of about two weeks at 22°C, which makes it possible to rear the nematode in Petri-dish cultures in a laboratory environment (Fig. 6C). In the parasitic form the nematode parasitizes but does not kill the larvae of *S. noctilio*, and causes sterility in the female wasp. Infested females act as dispersal agents for the nematode via their parasitized eggs. These attributes of *D. siricidicola*, together with its high specificity and parasitism rates, make it an ideal biological control agent.

Shortly after the detection of *S. noctilio* in Cape Town, efforts were made to introduce the Kamona strain of *D. siricidicola* (Tribe 1995, Tribe and Cillié 2004). In 1995, 250 *S. noctilio*-infested trees were inoculated with 70 million nematodes, and in 1996, 46 *S. noctilio*-infested trees were inoculated with 20 million nematodes (Tribe and Cillié 2004). Inoculations were done on *P. radiata* trees within a 90 km arc of

Cape Town, an area demarcating the boundary of the known occurrence of S. *noctilio* at that time. The standard inoculation method, described in Bedding and Iede (2005) was used. The nematodes were imported directly from Australia. In addition to these inoculations, in 1998 and 2002 *S. noctilio*-infested logs, where *D. siricidicola* parasitism had been confirmed, were transported to *P. radiata* plantations further east, as far as George, with the intention that infected *S. noctilio* females would spread the nematode in that area (Tribe and Cillié 2004). No inoculations were done in the Western Cape from 1997 to 2003.

Results of the nematode inoculations in the Western Cape were reported to be good, although variable. Tribe and Cillié (2004) recorded parasitism of 96.1 % within the inoculated area by 1998. But other studies have revealed more variable success in the province, ranging from 0-64 % (Hurley *et al.* 2007, data from 2001-2002 collections). More recent data reflect average parasitism levels of 53 % (author, unpublished data, based on adult female parasitism) to 64 % (Verleur and Kanzler (2007), based on larval scarring.

Prior to 2001, *S. noctilio* was thought to be well controlled in the Western Cape because it had not caused any serious mortality of pine. Consequently, the South African forest industry did not consider the wasps to be an imminent threat to the majority of the country's pine resources in KwaZulu-Natal and Mpumalanga. The perceived threat of *S. noctilio* increased when it was detected in Knysna in 2001 and then in Umtata, north Eastern Cape and KwaZulu-Natal in 2002 (Fig. 5). In 2003, *S. noctilio* was detected in various plantations in the Eastern Cape and KwaZulu-Natal and heavy infestations were observed. Unfortunately, neither *D. siricidicola* nor any other biological control agents of the wasp had apparently migrated northwards with *S. noctilio*. Again, *D. siricidicola* (Kamona strain) was seen as the most important biological control agent to release in these new epidemic areas, and the first releases started in 2004. This release required a re-negotiation with a private Australian company because the previous licence allowed release of the nematode only below 32°S latitude.

In 2004, a total of 1763 *S. noctilio*-infested trees were inoculated in the Eastern Cape and KwaZulu-Natal, with 178 million nematodes (Table 1). These inoculations were mainly in pulpwood compartments of *P. patula* where infestations were highest. Standard inoculation techniques (Bedding and Iede, 2005) were used. Success from these inoculations was poor, with only 3 % female parasitism obtained. Efforts were made to improve the inoculation success the following year. Particular emphasis was placed on inoculation technique and nematode survival during transport and in wood after inoculation. Operating practices involving nematode transport and inoculations were improved and preliminary studies showed that nematodes had survived well during transport at temperatures between 5-10°C and that they were penetrating and surviving in the wood. Despite these efforts, inoculations in 2005 again resulted in low levels of parasitism (Table 1).

Field trials were established in 2006 to test possible factors influencing success of *D. siricidicola* inoculations. Data from 2005 trials had indicated that the position (bottom, middle or top) of the inoculation in the trees affected inoculation success, which was thought to be due to differences in moisture content between these sections (Verleur and Kanzler 2006). These factors, as well as nematode source (those reared in South Africa as opposed to nematodes sourced from Australia), period of inoculation and inoculation method (conventional method of inoculating felled trees compared to inoculating standing trees) were examined (Hurley *et al.* 2008, Verleur and Kanzler 2008a). The inoculation of standing trees was tested as this targeted the bottom sections of the trees, where inoculation success was highest, was safer and more cost and time effective than having to fell trees for inoculation. Background parasitism, defined as natural spread and establishment of *D. siricidicola*, was also examined.

Results from the 2006 trials confirmed that inoculation success was highest from the bottom section of the tree and in earlier inoculation periods, where moisture content was highest (Hurley *et al.* 2008, Verleur and Kanzler 2008a). However, the correlation between moisture content and parasitism was low; indicating that moisture content was not the only factor influencing inoculation success. Inoculation of standing trees was more cost effective and safer (see Fig. 7). Nematode source was found not to influence inoculation success. Background parasitism was detected at the trial sites, from just over 1 % (Hurley *et al.* 2008) to about 20 % (Verleur and Kanzler 2008a). These results were very encouraging as they showed that despite the low inoculation success (7.2 %, Table 1), the nematodes had, at least to some degree, become established in the plantations.

Mass inoculations of *D. siricidicola* in the Eastern Cape and KwaZulu-Natal were planned for 2007 and 2008. This project was motivated mainly by the evidence of background parasitism, which meant that inoculations, even with low success, could spread the nematode across areas infested with *S. noctilio*, allowing the nematode population to naturally increase over time. Over these two years, 96 063 trees were inoculated with about 7.8 billion nematodes (Table 1). The majority of inoculations were in pulpwood stands where standing trees were inoculated. In saw timber stands, the conventional method of inoculating felled trees was followed. Average female parasitism was 22 % and 35 % from the 2007 and 2008 inoculations, respectively (Table 1). Although the sampling technique for the results in 2007 and

2008 included a higher proportion of bottom logs (where parasitism was higher), the results still showed a general improvement from the initial inoculations in 2004 and 2005, and showed an increase in parasitism over time. The majority of the increase could be attributed to the natural spread of the nematode, as inoculation success remained poor. This was evident from sites receiving their first inoculations where parasitism results were below 10 % (authors, unpublished data). Inoculations continued in 2009, but at reduced levels due to the decrease in infestations of *S. noctilio*. In total, 8 300 trees were inoculated in the Eastern Cape and KwaZulu-Natal with 830 million nematodes. Results from these inoculations were not available at time of writing this review.

The reason for the decline in *S. noctilio* infestations is not known for certain. Increasing levels of parasitism by *D. siricidicola* could have contributed to the decline, but because parasitism has only recently increased and is still not near the over 90 % infestation levels reported by Bedding and Iede (2005), especially from the middle and top sections of the trees, this may not be the main or only cause. Other possible factors include the decreasing supply of suitable hosts, with many suitable hosts previously attacked and killed by *S. noctilio* or removed by clear-felling of highly infested stands (see plantation management section).

Biological control: Parasitic wasps

Parasitic wasps are considered a key component for the control of *S. noctilio* (Taylor 1976). Nine species were released in Australia and of these *Megarhyssa nortoni*, *Ibalia leucospoides* and *Rhyssa persuasoria* were considered the most effective (Taylor 1978, Neumann *et al.* 1987). *Ibalia leucospoides* and *M. nortoni* were introduced into South Africa, but only *I. leucospoides* has become established (Tribe and Cillié 2004) (Fig. 6D).

In 1998, *Ibalia leucospoides* and *M. nortoni* were imported, from Uruguay and Australia respectively (Tribe and Cillié 2004). From the 18 male and 19 female *I. leucospoides* imported, 456 progeny were released from late 1998 to 2001. These parasitoids were released from Cape Town to Riversdale (Fig. 5). The establishment of *I. leucospoides* was not confirmed until 2002, but the parasitiod has now been detected in numerous plantations of the Western Cape. From these emerging wasps, a further nine *I. leucospoides* were released in Knysna in 2002. From the 10 male and 44 female *M. nortoni* imported in 1998, only 79 progeny were released the following year in Van Rhynsdorp (Tribe and Cillié 2004). No further releases were made and *M. nortoni* has since then never been recovered from the field, thus leading to the assumption that this biological control agent has not become established in South Africa.

Ibalia leucospoides appears not to have moved with *S. noctilio* as it spread to the Eastern Cape and KwaZulu-Natal. *Sirex noctilio* infested billets collected from Knysna in 2001, the Eastern Cape and Weza (near Kokstad) in 2002, and from various sites in KwaZulu-Natal from 2003 to 2006 indicated the absence of *I. leucospoides* in these areas (Fig. 5). Plans were thus made to introduce *I. leucospoides* to these areas. In January 2006, 138 *I. leucospoides* collected in the Western Cape were released at sites in KwaZulu-Natal and the Eastern Cape. A further 376 wasps were released from November 2006 to January 2007. *Ibalia leucospoides* were recovered from these release sites in late 2007 and released in the same area (a total of 201 wasps). In the subsequent *S. noctilio* emergence season, 74 *I. leucospoides* were recovered and these were released at sites in KwaZulu-Natal (Verleur and Kanzler 2008b).

Efforts are currently underway to develop a central rearing colony of *I. leucospoides*, from which the parasitoid can be sent to *S. noctilio* infested areas. This approach will provide a more sustainable and higher capacity to release the parasitoid than the capture and release method. A further aim is to increase the genetic diversity of the South African *I. leucospoides* population by introducing *I. leucospoides* from other countries into the rearing colony. The current genetic diversity of the South African population is expected to be very low due to the small number of wasps originally introduced. The influence of genetic diversity on the success of biological control agents is not clear, but could affect its ability to adapt to new environments and host types (Roderick and Navajas 2003).

Plantation Management

Stressed trees are known to be more susceptible to attack by *S. noctilio* (Madden 1968, Talbot 1977, Neumann and Minko 1981), and poor silviculture practices can be a major source of stress in plantations (Madden 1988). In South African pine forests, high stocking of pulpwood stands is likely one of the major reasons for the high levels of infestation of *S. noctilio* in regions where such stands are dominant. These stands are intentionally managed to have high stocking, with no thinning and no pruning or pruning only to allow access into the plantations. The goal here is to maximize revenue by maximizing fibre levels per hectare. The forest industry is currently investigating alternative management options for pulpwood stands, involving different thinning regimes in an effort to decrease stress in the stands while retaining economic feasibility. Saw timber stands are less prone to stress due to thinnings, when these are conducted at the optimal time.

One of the management strategies of the forestry industry in response to *S. noctilio* infestations has been to clear-fell and harvest areas where infestations are above 15 %. The harvested timber is moved to a mill where it was processed and the *S. noctilio* larvae destroyed. This strategy was used in KwaZulu-Natal, and likely contributed to the noticeable decline of *S. noctilio* in those areas in subsequent years.

Monitoring of *Sirex noctilio*

Effective management of *S. noctilio,* including the release of biological control agents, must rest on a clear knowledge of the distribution of the pest. In South Africa, monitoring of *S. noctilio* after its detection in 1994 was mainly by means of field surveys. These monitoring efforts resulted in the detection of *S. noctilio* in the Eastern Cape and KwaZulu-Natal (Fig. 5). Field surveys to detect *S. noctilio* in the northern Eastern Cape and KwaZulu-Natal started in 2002 after *S. noctilio* was unexpectedly detected in Umtata during a routine field excursion. The spread of *S. noctilio* to Umtata, far from the known range of *S. noctilio* the previous year, could have resulted from human-mediated transport of *S. noctilio*, but could also have resulted from the undetected movement of *S. noctilio* before 2002.

From 2004 onwards, various traps and lures based on volatile compounds from pine trees were tested for trapping *S. noctilio*. This work was conducted in collaboration with USDA-APHIS. These tests have resulted in the current black intercept (panel) trap, using a six-component blend lure containing general pine volatiles (Fig. 6E). Using this trap, it has been possible to detect new infestations of *S.*

noctilio and this also led to the detection of *S. noctilio* in northern KwaZulu-Natal during 2007 and 2008 (see Fig. 5) and more recently in Mpumalanga (November 2009). Trials are underway to improve the current trap type and lures used to monitor the spread of *S. noctilio*. These new traps using generic or specific lures will be used to detect the presence of other wood borers.

In 2004, the use of trap trees was tested to monitor the spread of *S. noctilio* for the first time in South Africa. This is an approach described by Madden (1971), where trees are stressed to lure *S. noctilio* females. Trap trees can function as a means of detection as well as an inoculation source for *D. siricidicola*. The trap trees set in 2004 were established using previously described techniques (Anon 2002, Neumann *et al*. 1982), except that glyphosate was used as an alternative to dicamba, which is prohibited for use in South African plantations. Success with these trap trees was very poor. Because there were high numbers of naturally infested trees to inoculate and because of the current development of lure-based traps to detect new *S. noctilio* populations, efforts to use trap-trees were abandoned until 2007. A decision was then made to re-investigate trap trees as a method for monitoring, and to complement the lure-based traps. These trees could then also be used to provide an inoculation source in areas with new *S. noctilio* infestations, and where naturally infested trees were difficult to locate. Trap tree trials in 2007, showed that trap trees could be successfully used when applied a month before adult emergence using Dicamba/24-D/MCPA (Verleur and Kanzler 2009). Plans are underway to use traps and trap trees on a larger scale in areas ahead of the known limits of *S. noctilio* distribution.

Conclusions

It is clear that local adaptation of control strategies for *S. noctilio* is required where this pest has entered new environments. In South Africa, after the detection of *S. noctilio*, control strategies developed in Australia were rapidly adopted, specifically the introduction of biological control agents. Early failures with biological control underscored the importance of developing and modifying control strategies that are effective under local climate, environmental and management conditions, as these factors influence tree vigour and, therefore, susceptibility to *S. noctilio*.

Amongst the major adaptations to the *S. noctilio* control programme in South Africa, those pertaining to the application of the parasitic nematode *D. siricidicola* have been most significant. This nematode was highly successful as a biological control agent in other parts of the world, yet gave poor results in the first two years of inoculation in the summer rainfall area of South Africa. Investigations revealed that low moisture content in the wood at time of inoculation had influenced success. This situation could not be completely avoided, but inoculation techniques were adapted to increase the efficacy of inoculations by targeting the wetter bottom sections of standing trees in pulpwood stands.

Other possible factors influencing inoculation success include the influence of different strains of *A. areolatum* present in South Africa and Australia (Slippers *et al.* 2001, author, unpublished data). Preliminary results have shown that the fungus associated with *S. noctilio* in South Africa may grow slower than the fungus on which *D. siricidicola* has been developed. The influence that this has on nematode survival and reproduction is being investigated. Furthermore, the competition between blue stain fungi commonly present in trees infested with *S. noctilio*, and *A. areolatum*, and the influence that this has on nematode survival is being considered. Both of these factors, namely *A. areolatum* strain and the presence and composition of blue stain fungi in infested trees, can differ between and even within countries. In addition, plans to collect and screen other strains of *D. siricidicola* for their efficacy in South Africa's summer rainfall region are underway.

Insufficient effort has been made to introduce parasitic wasps for biological control of *S. noctilio* in South Africa. These wasps can play an important role in the control of the pest, and could be especially important given the low inoculation success with the nematodes to date. Increased effort is needed to establish a sustainable rearing colony of *I. leucospoides* from which large numbers of wasps can be released annually. Genetic diversity of the population of the parasitic wasps must also be considered. In addition, effort is needed to import and release other parasitic wasps, such as *Megarhyssa nortoni* and *Rhyssa persuasoria*. There is little question that the introduction of these parasitic wasps will contribute to a more robust and sustainable biological control effort.

Plantation management is a major factor determining infestations of *S. noctilio* and requires further attention by the South African forest industry. Clear-felling of highly infested sites provides an effective management approach, but the vast areas of highly stocked, stressed pulpwood stands will always provide an opportunity for *S. noctilio* and other stress-associated pests and diseases to establish. Management strategies are governed by economics, but the financial implications of pest outbreaks need to be considered in this equation. The current thinning trials aimed at finding a 'middle road' are an indication that the industry in South Africa is taking pest threats very seriously.

Effective monitoring tools were developed a decade after *S. noctilio* was first detected in South Africa. Yet the development of monitoring tools should be a priority for any new pest introduction. It is clear that if greater attention had been paid to monitoring after the detection of *S. noctilio* in South Africa, its movement into the Eastern Cape and KwaZulu-Natal would have been recognized earlier, which would have resulted in earlier management interventions and many thousands of trees might possibly have been saved. The monitoring of *S. noctilio* continues to be crucial as it moves northwards.

The extent to which *S. noctilio* will infest plantations in Mpumalanga and the Northern Province provinces is currently unknown. This region has a mixture of saw timber and pulpwood stands and other stress factors such as baboon damage (McNamara 2006) will also need to be taken into account. Past efforts to adapt control strategies for local conditions will certainly contribute to ensuring low populations of *S. noctilio*. *Deladenus siricidicola* is already present at the front of the *S. noctilio* distribution and with the assistance of monitoring tools it can be rapidly introduced, together with *I. leucospoides*, to new infestation sites. Thinning overstocked stands ahead of the front and clear-felling highly infested sites should also be considered. Continued efforts to improve inoculation success, including research on possible barriers to inoculation, the introduction of new nematode strains and the introduction of other parasitic wasps will also be important. Progress in these areas will be fundamental to the future management of *S. noctilio* in South Africa.

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Table 1. Inoculations of *D. siricidicola* in the Eastern Cape and KwaZulu-Natal provinces of South Africa from 2004-2008.

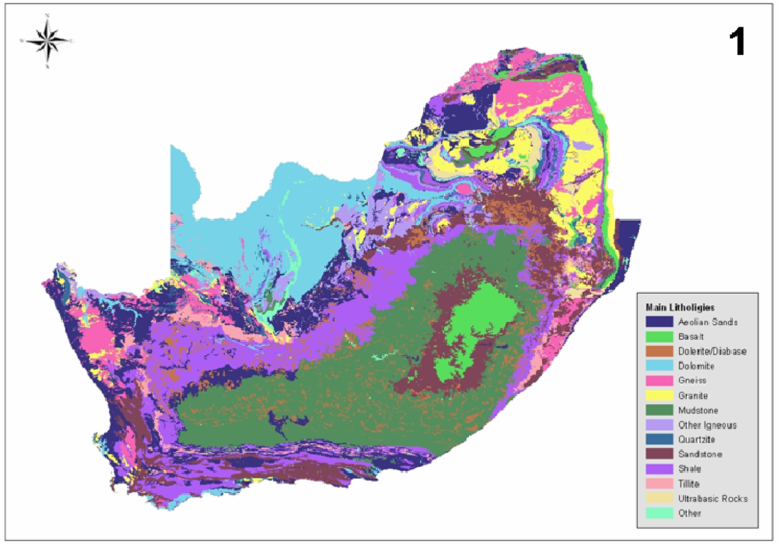
Year*	**Nematodes used (mil.)**	**Trees inoculated**	**% female parasitism of *S. noctilio*****
2004	178	1763	3 (*n* = 548)
2005	480	4400	7.8 (*n* = 1836)
2006***	40	396	7.2 (*n* = 2897)
2007	4641	57522	22 (*n* = 2132)
2008	3115	38541	35 (*n* = 1408)

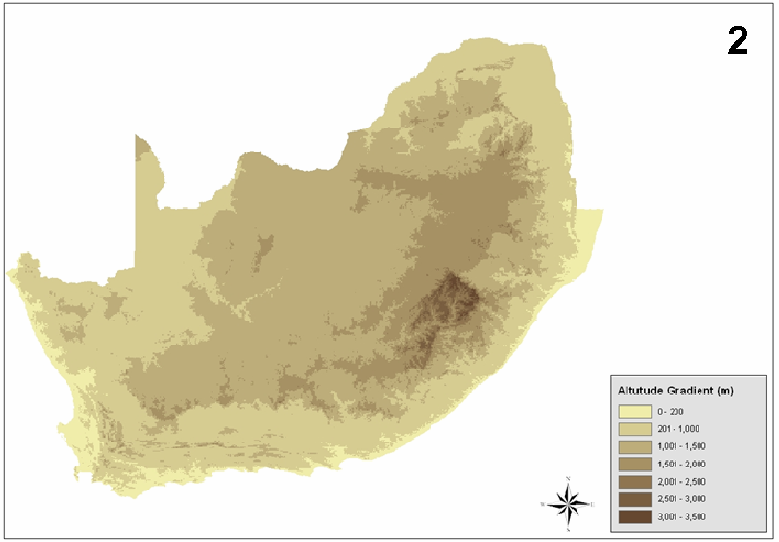
* In 2004-2005 felled trees were inoculated, while in 2007-2008 mainly standing trees were inoculated (fewer nematodes are used per tree). Comparison of parasitism results between years requires caution as different sampling strategies were used, where a greater proportion of bottom logs (where parasitism was highest) was sampled in 2007 and 2008.

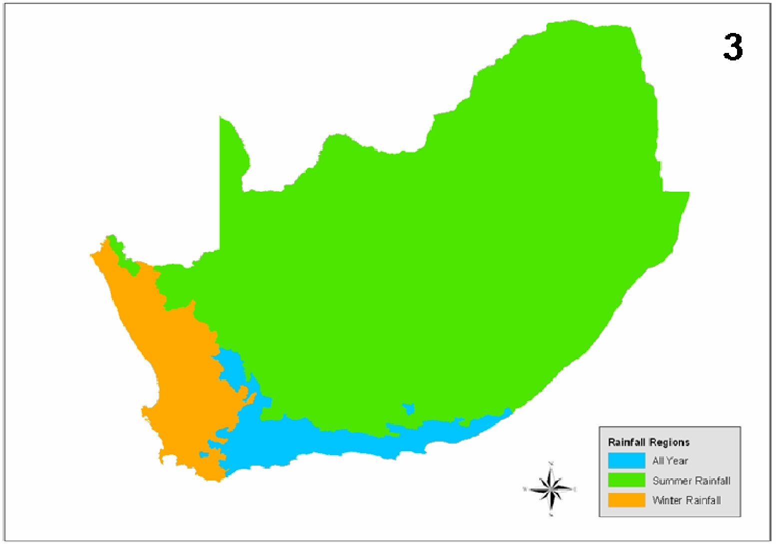
** *n* is the total number of female wasps dissected from sampled logs.

*** Inoculations in 2006 were part of a trial. All commercial inoculations were stopped in this year pending the result of the trial.

Figures 1-4. Maps of South Africa. 1. Lithology. 2. Altitude. 3. Rainfall. 4. Distribution of pine forestry. (Data sources: Schulze (2007))


1
Main Lithologies
Aeolian Sands
Basalt
Dolerite/Diabase
Dolomite
Gneiss
Granite
Mudstone
Other Igneous
Quartzite
Sandstone
Shale
Tillite
Ultrabasic Rocks
Other


2
Altutude Gradient (m)
0 - 200
201 - 1,000
1,001 - 1,500
1,501 - 2,000
2,001 - 2,500
2,501 - 3,000
3,001 - 3,500


3
Rainfall Regions
All Year
Summer Rainfall
Winter Rainfall

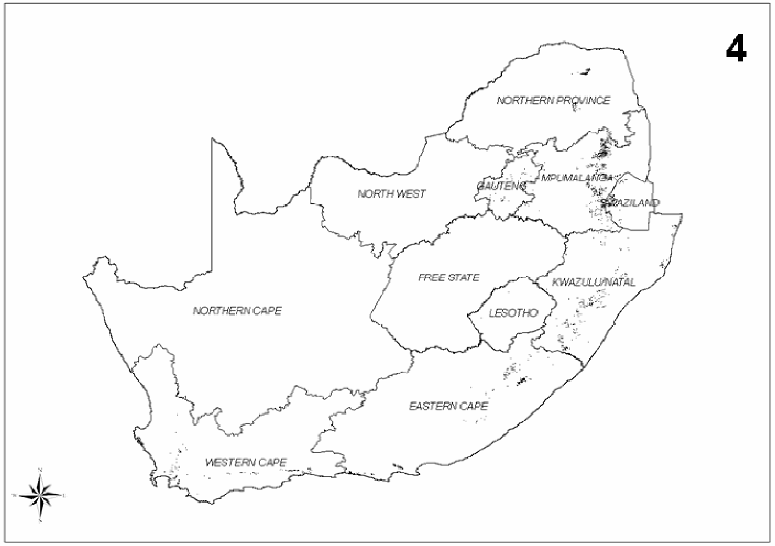
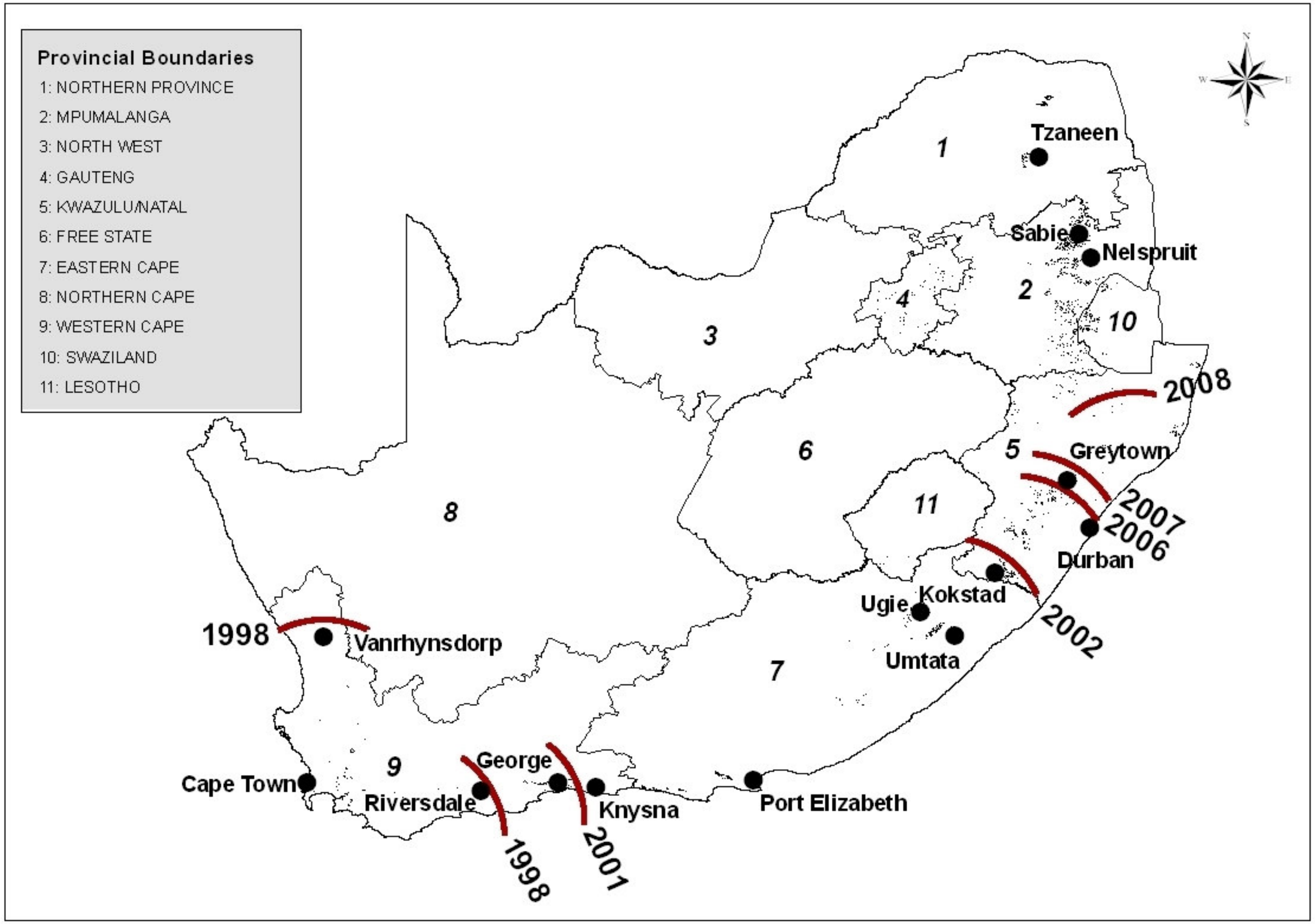

4
NORTHERN PROVINCE
NORTH WEST
GAUTENG
MPUMALANGA
SWAZILAND
FREE STATE
KWAZULU/NATAL
LESOTHO
NORTHERN CAPE
EASTERN CAPE
WESTERN CAPE

Figure 5. Spread of *S. noctilio* in South Africa from 1994-2008


Provincial Boundaries
1: NORTHERN PROVINCE
2: MPUMALANGA
3: NORTH WEST
4: GAUTENG
5: KWAZULU/NATAL
6: FREE STATE
7: EASTERN CAPE
8: NORTHERN CAPE
9: WESTERN CAPE
10: SWAZILAND
11: LESOTHO
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Tzaneen
Sabie
Nelspruit
Greytown
Durban
Kokstad
Ugie
Umtata
Vanrhynsdorp
Cape Town
Riversdale
George
Knysna
Port Elizabeth
2008
2007
2006
2002
2001
1998
1998

Figure 6. A. *Sirex noctilio* male emerging from untreated wooden packing material bores through empty cement bags sent to Johannesburg, showing ease at which *S. noctilio* can spread within South Africa. B. *Pinus patula* trees killed by *S. noctilio* in the KwaZulu-Natal province. C. The nematode *D. siricidicola* growing on the fungus *A. areolatum* on Petri-dish cultures in a laboratory environment. D. The parasitoid wasp *I. leucospoides* released as a biological control agent for *S. noctilio*. E. Lure-based black panel traps used to detect new infestations of *S. noctilio*.

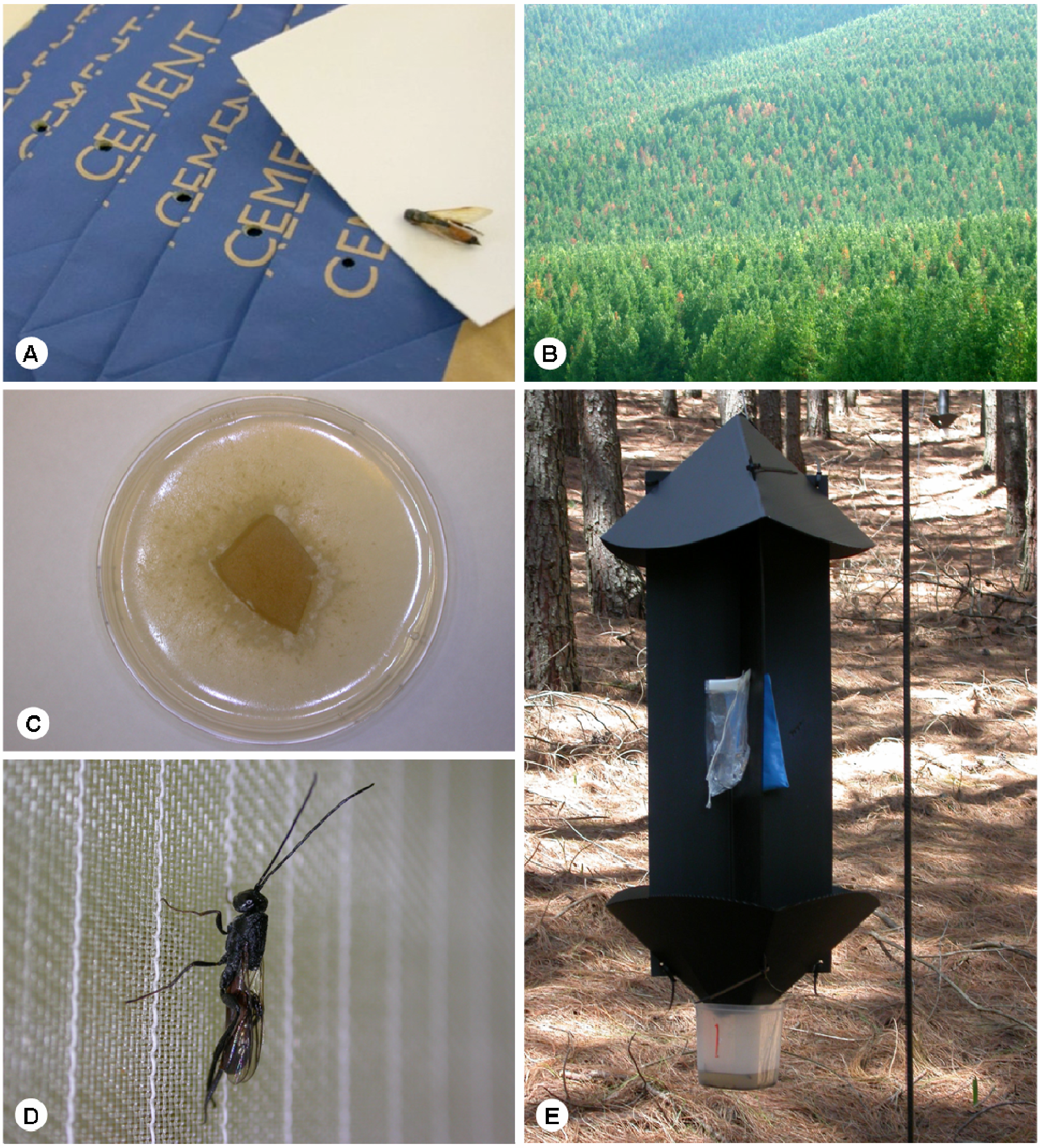
A
B
C
D
E

Figure 7. Techniques used to inoculate *D. siricidicola* into *S. noctilio* infested trees. A, B, C. Standard procedure of felling trees to inoculate. D. Inoculation hammers used. E. Adapted procedure of inoculating standing trees (used in pulpwood stands).


A
B
C
D
E