Chapter 9
Factors Affecting the Efficacy of *Deladenus siricidicola* in Biological Control Systems

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Abstract The nematode, *Deladenus* (=*Beddingia*) *siricidicola*, represents the cornerstone of *Sirex noctilio* biological control programs across the Southern Hemisphere. There is, however, significant variation in its efficacy in different regions. In this review, we consider emerging issues related to the biology and handling of the nematode that might influence its efficacy in biological control systems. Most practical aspects concerning the handling of *D. siricidicola* have been streamlined over the past half-century and these appear to be very efficient. However, large gaps remain in our knowledge about some key aspects of the biology of *D. siricidicola*. For example, very little is known regarding the evolution of virulence in the nematode populations, and the consequent evolution of resistance in *S. noctilio* populations. Furthermore, the levels of diversity in *D. siricidicola* and its ability to adapt to fungal, wasp and environmental variation are poorly understood. In this regard, new collections and storage of native populations of the *Deladenus* spp. are critical for the future research and management of this key biological control agent of *S. noctilio*.

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9.1 Introduction

Sirex noctilio was accidentally introduced into New Zealand around 1900 (Miller and Clark 1935). The wasp soon became a serious pest in *Pinus radiata* plantations and during the 1940s and 1950s reached epidemic proportions (Rawling 1955). The damage caused by the wasp sparked intensive studies on its biological control, initially focussed on parasitic wasp species (Hanson 1939; Nuttall 1989). During the course of this work, a nematode-infected female *S. noctilio* was discovered on the north island of New Zealand (Zondag 1962). The nematode was thus naturally introduced together with *S. noctilio* into this region. It soon became clear that this nematode held promise as a biological control agent (Zondag 1965, 1967, 1969, 1971, 1979). This stimulated the emergence of a research field that continues today and that has made *D. siricidicola* one of the best-studied entomopathogenic nematodes in any system.

Deladenus siricidicola has a bi-cyclic life cycle (Fig. 9.1), including a mycetophagous or free-living and a parasitic cycle (Bedding 1967). The two morphological forms associated with this unusual life history are so distinct that it might initially have been described in two families, the Neotylenchidae (where it is currently placed) and the Allantonematidae (Bedding 1967, 1974). In the free-living cycle, the nematode feeds exclusively on *Amylostereum* spp., the fungal symbionts of Siricid wasps, and it reproduces oviparously (Bedding 1967, 1972). In the parasitic cycle, female nematodes enter and develop in the haemocael of the Siricid larvae and reproduce ovoviviparously (Bedding 1967, 1972). Nematode larvae produced by parasitic females are released inside the haemocael of Siricid larvae, and migrate towards and then infest the testes and developing eggs.

Deladenus siricidicola sterilizes the female of *Sirex noctilio*. The nematode does not affect oviposition and is consequently spread by the female wasps through infected eggs (Zondag 1969; Bedding 1972). Furthermore, the free-living cycle makes it possible to rear the nematode in large quantities in the laboratory and thus to be able to achieve mass releases in the field (Bedding 1974; Bedding and Iede 2005) (Fig. 9.1).

To date, seven *Deladenus* spp. have been described associated with the Siricid-*Amylostereum* symbiosis (Bedding 1974). Several of these species also infest the parasitoids of Siricids and the beetle *Serropalpus barbatus* (Bedding 1967, 1972, 1974). In addition, *D. siricidicola* is highly specific to Siricids and *A. areolatum*, including not infesting other hymenopteran parasitoids of Siricids (Bedding and Akhurst 1974, 1978). These characters together have made *D. siricidicola* an ideal biological control tool. Initially it was introduced between plantations by moving infested logs , wasps or contents from infested wasps (Zondag 1969, 1971). Subsequently, artificial inoculation has become the preferred method of introduction into plantations, followed by natural spread by female wasps. This followed intensive work on artificial rearing of the nematode on *A. areolatum* cultures (Bedding and Akhurst 1974; Bedding 1979), and subsequent development of the method to inoculate it into cavities punched into tree stems together with a carrier gel solution (Bedding and Iede 2005).

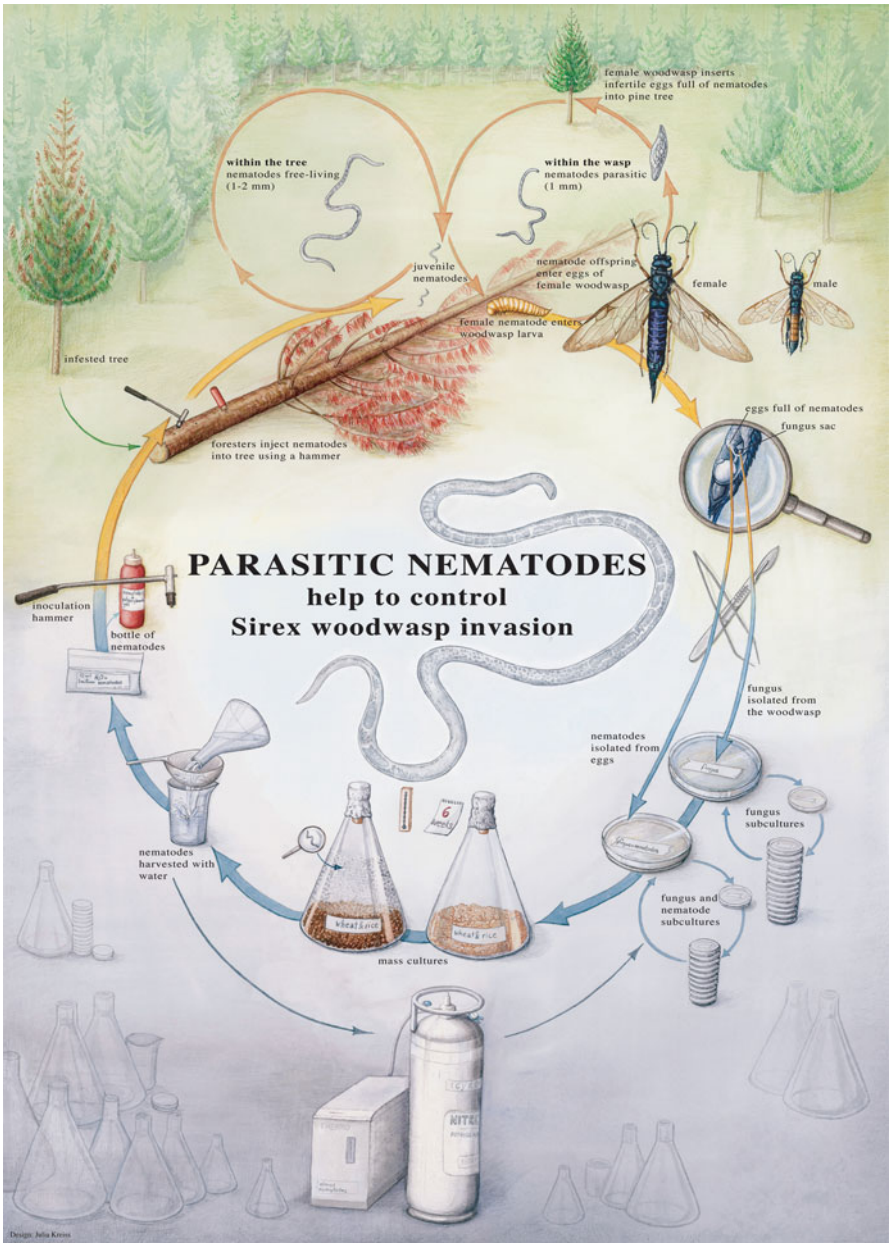

Fig. 9.1 *Deladenus siricidicola* mass rearing and release strategy and bi-cyclic life cycle. The image was produced by Julia Kreiss

Today, *D. siricidicola* is considered to be the cornerstone of ongoing biological control programs against *S. noctilio* in Australia, South America and South Africa. In New Zealand the nematode is no longer actively inoculated into trees, but it still contributes to the control of the wasp together with parasitic wasps (John Bain, personal communication 2010; Chap. 13). The methods to produce and inoculate *D. siricidicola* vary from region to region, but generally follows the basic principles described during the 1970s to 1990s in Australia (Fig. 9.1).

Biological control using *D. siricidicola* is not equally successful in all regions of the world, with inoculation success varying from <5% to >99% in different regions (Hurley et al. 2007). The factors affecting this variation in success have, however, not been widely studied. Hurley et al. (2008) attempted to identify a number of potential factors affecting *D. siricidicola* in summer rainfall regions of South Africa. The conclusions derived from this study were that a combination of factors including moisture in the wood, virulence, resistance, competing micro-organisms, and variation in the *A. areolatum* strain used for inoculation could contribute to inoculation success.

Many excellent reviews treating the biology, history and use of *D. siricidicola* for the biological control of *S. noctilio* have been published. For example, Bedding (1979), Neumann et al. (1987) and Bedding and Iede (2005) reviewed the biology, development and application of *D. siricidicola* in field management systems. In a recent review, Hurley et al. (2007) and Chaps. 14–18 considered programs in the Southern Hemisphere where *D. siricidicola* is being used for the control of *S. noctilio* and particularly focussed on the relative success of these programmes. The aim of this chapter is to consider emerging issues related to the biology and handling of the nematode that might influence its use in biological control systems.

9.2 Rearing, Handling and Storage

The success of *D. siricidicola* as a biological control agent requires effective methods to mass-rear, release and store the nematode. Developments in these areas (as described in Bedding and Iede 2005; Fig. 9.1) have contributed substantially to the feasibility of using this nematode as a biological control agent effectively. This has also promoted its introduction into all Southern Hemisphere countries where *S. noctilio* is present. However, the use of *D. siricidicola* is not without challenges, and poor quality control in the rearing, handling and storing of *D. siricidicola* can drastically decrease *S. noctilio* control.

Contamination of the medium used to grow *A. areolatum*, the fungal food source for *D. siricidicola,* can result in greatly reduced numbers of nematodes produced. This is exacerbated by the fact that the nematode cultures take many weeks to mature. In worst cases, the majority of a rearing population can be lost in a short time. The contaminants, including bacteria and mites, are often transferred by mites and can spread rapidly within a rearing facility. It is thus imperative to ensure that working conditions are as clean as possible. However, even under the sterile conditions,

contamination is likely to occur during the mass rearing process and this needs to be considered when planning control programs.

The temperature of the nematodes during their transit to the field is a major factor influencing their survival. Nematodes are initially transported in water in sealed, breathable plastic bags and later suspended in a polyacrylamide gel before being inoculated into the trees, typically under cool conditions (<5°C) (Bedding and Iede 2005). Recent studies examining the survival of nematodes in water have confirmed that nematode survival decreases over time and as temperature increases (BP Hurley, unpublished data). For example, at 5°C and 10°C, over 80% of the nematodes survived after 150 h, whereas at 25°C only 39% survived after 30 h, and at 30°C only 7% survived after 24 h. Similarly, nematode survival in the polyacrylamide gel has also been shown to decrease with an increase in temperature (authors, unpublished data). The temperature of the gel is, however, less of a concern as the nematodes generally remain in the gel for only a short time before being inoculated into trees. In contrast, the nematodes can remain in the water-filled bags for numerous days. This increases the chance for them to be exposed to high temperatures and this can consequently greatly reduce the success of the inoculations.

A specially designed rebound hammer punch is used to inoculate trees with *D. siricidicola* (Bedding and Iede 2005, Chap. 14). The hammers are designed to make clean holes in the wood without bending the tracheids, thus allowing entry of the nematodes into the wood. Nematode numbers introduced into trees are significantly reduced when blunt punches are used (BP Hurley, unpublished data). Furthermore, the level of care with which contractors use the hammer punches to produce inoculation holes can also influence the quality of the inoculation site and thus nematode establishment.

Continuous rearing of the mycetophagous stage of *D. siricidicola* on *A. areolatum* over a number of years can result in a loss of virulence of the nematode (Bedding and Iede 2005). This can have serious negative consequences for biological control efforts, as was observed in the Green Triangle of Australia (Haugen 1990; Haugen and Underdown 1990; Haugen and Underdown 1993). This problem can be largely solved by storing nematodes in liquid nitrogen outside of the inoculation season (Bedding and Iede 2005).

9.3 Evolution of Nematode Virulence and Wasp Resistance

Variation and natural change in resistance of the *S. noctilio* populations to infection by *D. siricidicola*, and equally, changes in the virulence of *D. siricidicola* populations should be expected over time. Such variation and change are common patterns in biological interactions and they are thought to be linked to the evolution and maintenance of sexual reproduction in biological systems. The red queen hypothesis (van Valen 1973) postulates that hosts and their parasites are in a continual “arms race” involving cycles of evolution of resistance (including tolerance) in the host and over-coming resistance (including higher levels of virulence) in the parasite.

The "trade-off" hypothesis between transmission and virulence predicts that parasites will evolve towards lower levels of virulence in situations where there is a restriction on spread linked to high levels of virulence (Alizon et al. 2009). This idea has been most intensively explored in human-pathogen interactions, but not in agricultural or forestry situations. It is especially relevant to some biological control systems, in particular classical biological control, such as the *S. noctilio-D. siricidicola* system, which relies on the natural dispersal of the parasite that is often linked to the dispersal of the host.

Virulence is defined here as the number of adults in a given *S. noctilio* population that are infested by *D. siricidicola* and are sterilized by it. In female wasps this only includes individuals with infested eggs. Usually all eggs in such females will be infested. The nematodes sometimes infest the females, but they do not enter the eggs (Bedding 1972, 1974; Zondag 1975; Yu et al. 2009). For the purposes of this discussion on nematode virulence, these female wasps are not sterile and are thus not included in counts of infected wasps in a population.

It is not known how effectively *D. siricidicola* can spread if it does not infect the eggs. How this condition influences the fitness of the nematode populations is thus not known. This is an important question to answer, especially because the condition appears to be common in some regions. Bedding (1972, 1974) reported this condition in a number of populations. The latter studies also reported this to be the case for *D. imperialis* and *D. rudyi*. Yu et al. (2009) found that none of the 102 nematode-infected *S. noctilio* females collected from various sites in Canada had infected eggs. Zondag (1975) also noticed nematode infected *S. noctilio* females, where the eggs were not also infested. The possibility that the nematodes can spread without infecting the eggs is suggested by the fact that they can be found in the oviducts of the female wasp through which the eggs will pass during oviposition. If there is no selective advantage to *D. siricidicola* spread via eggs and thus sterilizing the host over spreading without infecting the eggs, then it is hard to imagine why all populations would not exclusively spread outside the eggs. Furthermore, if this condition is genetically controlled, then cross-breeding between infective and non-infective strains of *D. siricidicola* should be vigorously avoided where *S. noctilio* control using this nematode is important. There is thus a critical need to better understand the mechanisms that underlie and drive the ability of the nematode to infect Siricid eggs. Futhermore, variation in this condition should be compared in the different regions of the world where *D. siricidicola* is being used for biological control.

Bedding (1974) noted that there is a direct correlation between time of release of juvenile nematodes by infective female wasps, and subsequent infection of the host eggs. He also speculated that the nematode forms that do not enter eggs have evolved in host species that are more solitary. This is in contrast to wasps that often infest the same trees that would allow the evolution of highly virulent, egg-infecting nematode forms, because non-infected hosts are likely to also oviposit in the same tree (Bedding 1972, 1974). Bedding and Iede (2005) report high levels of virulence even under very low levels of plantation infestation (<1%) where attacks are very sparse. There are, however, no other data to make an evaluation of this hypothesis possible.

The infection of wasps by *D. siricidicola* is expected to have an effect on the population of *S. noctilio* even if the nematode does not infect the eggs. A number of researchers (Zondag 1969; Bedding and Iede 2005; Corley and Villacide 2005) have noted that infection by *D. siricidicola* leads to lowered fat reserves in the larvae and adults and that this affects their size and ability to fly. Their fitness would thus be negatively affected, in terms of numbers of eggs produced, dispersal distance and energy to oviposit. The effect of wasp infection without entering the eggs on the fitness of the *S. noctilio* population, however, cannot be quantified at present.

Zondag (1969) noticed that heavily infected wasps sometimes have no fat bodies remaining and they were often observed to die. He stated that 'The most important deviation from the normal pattern of the nematode infection is that the hosts can die when the immature hosts are heavily infected.' Bedding (1972) disagreed with this view and concluded that heavy infestation by the nematode does not lead to the death of the larvae. This is an important question as it underpins a potential driving mechanism for the evolution towards lower virulence in *D. siricidicola*, which would emerge where inordinately high levels of virulence in the nematode increase mortality in the larvae. This could then also be affected by levels of artificial inoculation, where heavy inoculation might lead to high infection levels and high larval mortality. The consequence would be that nematodes that are less virulent and consequently kill less larvae would survive. In this regard, Bedding and Akhurst (1974) noticed that heavily inoculated logs (at 10 cm intervals) produce smaller and fewer numbers of females, seemingly confirming this view.

Mechanisms of natural resistance in *S. noctilio* populations, other than the non-infection of eggs described above, might also exist. For example, Bedding (1972) noticed that nematodes, which infect and sterilize the Australian population of *S. noctilio*, do not do so in the Belgian population of *S. noctilio* where it originated. It is also obvious that any resistance that arises by mutation in a particular population of *S. noctilio* is likely to become fixed rapidly, especially under the strong selective pressure that would emerge where nematode infection sterilizes all non-resistant individuals. The mechanisms that might drive such resistance are not understood and only open to speculation. One possibility might be that resistance to infestation by *D. siricidicola* involves co-ordination of the development of eggs and the nematodes. The nematodes must enter the egg at a very specific stage of development, or they will either stop egg development completely or fail to penetrate the eggs (Bedding 1972). This is clearly an issue that needs urgent consideration.

Another source of variation in virulence in *D. siricidicola* might be by the loss of the ability to develop infective females due to continuous artificial rearing in the free-living cycle in the laboratory (Bedding 1972). The continual selection of those individuals that develop into mycetophagous forms (as opposed to infective females) might lead to such a loss. This is thought to have been the cause of the collapse of the biological control system in Australia during the 1987 to 1990 outbreak in Victoria (Haugen and Underdown 1993). The nematode that had previously consistently resulted in infection levels of above 95% after inoculation, then infected less than 30% of the wasp population after inoculation. To overcome this problem, a new and virulent nematode strain was re-isolated, which again brought parasitism levels

after inoculation to >90% (Bedding and Iede 2005). There is, however, no direct evidence that the loss of virulence was caused by this rearing process. Attempts to produce a non-infective strain, for research purposes, by continuously rearing the nematode artificially for the past 6 years at the University of Pretoria, has failed to re-create this effect (Hurley et al. 2008 and subsequent work). The underlying reasons why *D. siricidicola* lost its virulence in Australia and Brazil remain unclear and this could be due to one or more of the factors discussed previously in this review.

9.4 Introduction History and Genetic Diversity

In 1962, more than half a century after *S. noctilio* was first reported in New Zealand, Rudi Zondag noticed size changes in female and male reproductive organs of wasps from the Rotoehu forest (Zondag 1962). Upon closer inspection, this was found to result in nematode infection. Subsequently, nematodes were also described from the parasitoid wasp *Rhyssa* spp. in New Zealand and from various Siricids and their parasitoids from England (Bedding 1967, 1968a, b, 1972; Hocking 1967), including the description of the bi-cyclic life cycle of the species of *Deladenus*.

Subsequent to the discovery of *D. siricidicola* and the realization of the obvious potential it has for biological control, extensive surveys were initiated by the CSIRO (Australia). Ultimately thousands of logs and tens of thousands of wasps from across Europe, North Africa, various sites in North America, Japan, Pakistan and India were collected and screened for nematodes (Bedding 1972; Bedding and Akhurst 1974; Bedding and Iede 2005). These collections represented various species of *Deladenus*, as well as strains of *D. siricidicola* that produced lower levels of infection, or smaller wasps. After extensive screening of strains in the early 1970s, four strains from Corsica, Thasos, Sopron and New Zealand that gave high levels of infestation were selected for final trials. Of these, strain 198 from Sopron in Hungary was finally selected for wide scale application (Bedding and Iede 2005).

The Sopron strain of *D. siricidicola* has been the predominant strain inoculated throughout Australia since the early 1970s. However, it is expected that other strains were dispersed on a limited scale in early years of development of the biological control program in that country. Bedding (1972) reports that '…maintaining strains from many countries and already hundreds of millions of nematodes have been reared and distributed throughout many of the Sirex infested forests of Australia with encouraging results.' The nematode was not distributed in New Zealand and consequently its populations in that country are expected to still reflect original, accidental introductions of another strain(s). The 'Sopron' strain has subsequently also been used widely for inoculations in South America (Iede et al. 1998; Klasmer et al. 1998; Maderni 1998; Bedding and Iede 2005; Chaps. 15–17).

The Sopron strain of *D. siricidicola* has been reported to have lost its virulence in three cases, once in Australia in the late 1980s (Haugen and Underdown 1993; Bedding and Iede 2005), once in Brazil (Bedding and Iede 2005) and once in Argentina (Eskiviski et al. 2003, 2004). This reported to be due to repeated culturing of the

nematode for more than 15 years in the free-living form and consequently, its loss of ability to covert to the infective form, at least in culture (Bedding and Iede 2005).

In Australia, the loss of virulence in the Sopron strain was resolved by re-isolating the nematode from the Kamona forest in Tasmania, where original releases of the Sopron strain had been made years before (Bedding and Iede 2005). The culture was selected from a limited number of individual nematodes from a single tree. Subsequent inoculations resulted in high levels of virulence. This strain has subsequently been used extensively in the biological control program of *S. noctilio* in South Africa. In Brazil and Argentina, nematode strains were also isolated from infested wasps to establish new colonies (Eskiviski et al. 2003, 2004; Chap. 16). A strain resulting from these isolations and known as 'Encruzilhado do Sol' (Southern Hybrid), is widely used today in South American countries.

One of the consequences of introductions of *D. siricidicola* into Australia, South America and South Africa is that there is a lack of genetic diversity in populations of the nematode. A recent study has shown that the nematode populations from across this region are homozygous for 17 microsatellite regions and 3,291 bp of sequence data (Mlonyeni et al. 2011). This most likely result from a genetic bottleneck in the nematode population created during every round of sub-culturing. A selection of between 100 to 2,000 nematodes is typically transferred between plates and this process is often repeated numerous times. Furthermore, inbreeding levels would also be expected to be high in this system and this would be expected to rapidly reduce heterozygosity. This lack of diversity can be a problem, because the nematode is used in a variety of environments, and in different populations of the wasp and fungus. Its selection for a specific environment is expected to be high due to the strong human selection during isolation, rearing and inoculation.

9.5 Interaction Specificity – *Amylostereum* and *Sirex*

Specificity of *D. siricidicola* to specific wasp populations, and *vice versa*, has been observed. As discussed above Bedding (1972) and Bedding and Akhurst (1974) noticed that a strain of *D. siricidicola* from Japan never infects the eggs of *S. noctilio* females. Similarly, a strain that did not infect the eggs of females in the New Zealand population of *S. noctilio* was reported by Zondag (1975), alongside strains that could sterilize females in this wasp population. This reflects a process that is influenced by the particular strain of the nematode. In contrast, a strain of the nematode from Belgium sterilizes Australian populations, but not Belgian populations of the wasp. In the latter case, it is the wasp population that clearly influences the effect. The drivers behind these apparently strain specific interactions are not clear. Molecular tools now available should make it possible to better understand the relationships between different wasp and/or nematode populations. Irrespective of the driving forces behind host specificity in *D. siricidicola*, this factor clearly needs to be considered in *S. noctilio* management. This is especially because differences in wasp populations or invasions of new populations of the wasp can have far-reaching

consequences on the efficacy of biological control programs. This is especially relevant given the lack of diversity in the nematode populations discussed above.

The fungal strain that has been used to rear *D. siricidicola* in Australia (here referred to as the "nematode strain") is thought to have originated from early collections of wasps in Europe, possibly from *S. juvencus*. This strain has been shown to be distinct from the strain in the field in Australasia, South Africa and South America using VCG and molecular markers (Slippers et al. 2001, 2002; Nielsen et al. 2009). The "nematode strain" of the fungus is easily spread during the inoculation of the nematode, as harvesting of the nematodes from fungal cultures also contains many viable propagules of the fungus. This specific fungal strain is widely used across the Southern Hemisphere for mass rearing the nematode.

The difference between the "nematode strain" of the fungus and the strain of *A. areolatum* found in the field across the Southern Hemisphere is potentially important for biological control programs (Hurley et al. 2007). It has been observed that the nematode feeds and develops better on the "nematode strain" of the fungus than on other strains (Authors observations during mass rearing of the nematode). These preliminary observations could thus far not be quantified in experiments (BP Hurley, unpublished data). Nor could the nematode be selectively bred to reproduce more effectively on the field strains collected from *S. noctilio* in South Africa, despite multiple generations over a 2 year period (BP Hurley, unpublished data). Given the importance of potentially lower fitness of *D. siricidicola* on fungal strains other than the "nematode strain", this question needs to be urgently addressed.

9.6 Variable Environmental Factors

For many years it was assumed that *S. noctilio* populations performed best in Mediterranean, winter rainfall regions similar to its most common distribution in Europe (Kirk 1974; Spradbery and Kirk 1978). During the course of the last two decades the wasp has spread and prospered in winter and summer rainfall areas, in particular in Brazil and South Africa (Iede et al. 1998; Hurley et al. 2007). Projections based on current distribution also show that large parts of North and South America, Africa and Australasia would be suitable to future invasion by the wasp (Carnegie et al. 2006). From the South American and South African experiences in particular, it has become evident that the efficacy of the standard biological control programs developed in winter rainfall regions of Australasia, in particular with the nematode, will not be equally effective in all these regions.

Sirex noctilio populations are known to differ substantially in phenology in different climatic zones, which could affect the interaction with the nematode (Neumann and Minko 1981; Carnegie et al. 2005; Hurley et al. 2007). In New Zealand, the Cape region of South Africa and south-eastern pine-growing regions of Australia (Victoria, New South Wales), *S. noctilio* emergence is from December to April (peaking in February), while it occurs between October to January in the summer rainfall regions of South Africa (peaking in November).

One of the major differences between summer and winter rainfall regions that affect *D. siricidicola* is the rate of change in wood moisture after infestation. In the winter rainfall regions the majority of the time that the nematodes are in the trees is during the wet season, while in the summer rainfall regions the nematodes are in the trees mainly during the dry season. In the summer rainfall area of South Africa, moisture content of the trees, especially in the upper sections of the trunks, often drops below 20% (Hurley et al. 2007, 2008). It is not known what the threshold of moisture content is, below which the nematodes and/or the symbiotic fungus, *A. areolatum*, will survive, but lower moisture content has been linked to lower nematode parasitism (Hurley et al. 2008).

Recent studies have shown that some sap stain fungi compete strongly with *A. areolatum* for resources (authors, unpublished data). In particular, *Diplodia pinea*, a commonly occurring and important latent pathogen of pine in South Africa (Swart and Wingfield 1991), grows faster than *A. areolatum*, especially in environments of lower moisture availability. Although *D. pinea* has not been found to overgrow *A. areolatum*, its faster growth enables it to capture more wood resources and thus to limit the growth of *A. areolatum*. Where the growth of *A. areolatum* is severely limited, this will influence the survival and reproduction of *D. siricidicola*. Thus, the composition of sap stain fungi, and conditions that favour the establishment and growth of these fungi in an area, is likely to influence the successful establishment of *D. siricidicola*, and consequently effective biological control.

Various other factors vary between regions and could possibly influence the establishment of *D. siricidicola*, but their effects have not been studied. For example, many different *Pinus* species are planted in the regions where *D. siricidicola* is applied for biological control. These include *Pinus radiata*, *P. patula*, *P. taeda*, *P. carribea*, *P. pondersae*, *P. elliottii*, *P. contorta* var. *latifolia*, and hybrids of some of these species and some could be more suitable as hosts for *S. noctilio*, *A. areolatum* or *D. siricidicola*. Besides these factors that might influence variation, the effects of interactions between the above mentioned factors are also unknown. It is, for example likely that factors such as moisture content in the trees and the effect of sap-stain fungi would be correlated. Many smaller effects working in an additive fashion, as opposed to a single dominant effect, could also result in major differences in parasitism in control programs in different regions.

9.7 Conclusions

The discovery and description of the *D. siricidicola* in New Zealand and Australia represented a significant and exciting scientific breakthrough. The work that followed in this area during the subsequent decades led to the development of a biological control application that has saved potentially billions of dollars of damage to pine plantation industries across the Southern Hemisphere. Despite this success, there are regions where the nematode has not been as successful or has failed completely. As *S. noctilio* continues to spread throughout previously unaffected

areas, understanding the cause of this variation and improving the efficacy of biological control will be important. Information and interpretations provided in this review will hopefully clearly illustrate that much work remains to understand the causes of variation in efficacy of *D. siricidicola* in various parts of the world. Such understanding should make it possible to predict problems that will result in ineffective control and to ultimately also avoid these effects.

This review has treated many factors that could potentially affect the usefulness of *D. siricidicola* as a biological control agent. These include factors such as handling of the nematode, environmental factors, variation in the wasp and nematode populations, competing fungi and others. It seems unlikely from current evidence that a single factor is responsible for the variation in nematode success as a biological control agent. Rather, an interaction between several of these factors, or a number of factors in concert will more likely combine to cause the overall effect of dramatically different parasitism results.

It is unfortunate that variation, both molecular and phenotypic, in the populations of *D. siricidicola* has not been considered previously. The potential variation in factors such as the ability of the nematode to adapt to variable environments and populations of the wasp are all of great relevance to control strategies. Furthermore, the mechanisms driving evolution of resistance and virulence in the *D. siricidicola* and *S. noctilio* populations should be high on the research agenda for the future. Recently developed molecular markers should assist in this process. The potential to use rapidly developing novel approaches to DNA sequencing and thus to be able to analyse genetic and genomic factors underlying aspects such as virulence in *D. siricidicola* is great. Studies arising from the application of these technologies and others that have yet to emerge will surely improve our understanding of the biology of *D. siricidicola* and this will enable improved control.

Perhaps the greatest factor hampering research on *D. siricidicola* currently is a lack of availability of natural variation in available *D. siricidicola* strains. During the height of the collection and research programmes on *D. siricidicola* supported by the Australian and UK governments in the 1970s, numerous strains of this nematode (and its relatives) were collected from across the Northern Hemisphere. Today none of those strains from Europe remain available for study and it is only the Kamona strain, and recently strains collected in Canada, that can be investigated. Without appropriate investment in this aspect of the research on *D. siricidicola*, it should be expected that failures in biological control programmes will occur in the future and especially as a result of resistance emerging in populations of *S. noctilio*.

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