

**Monitoring the spread of *Sirex noctilio* in pine
plantations in South Africa (2004 – 2014)**

By

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A dissertation submitted to the Faculty of Science and
Agriculture in fulfilment of the academic requirements for the
degree of Master of Science

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2016

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Acknowledgements

The South African *Sirex* Control Programme (SASCP) Steering Committee members under the chair of Professor Colin Dyer for allowing me the opportunity to study and coordinate the SASCP operational section of the control programme.

Brenda Croft, my wife, for her encouragement and support.

Marcel Verleur, for all the work and dedication to the SASCP from its inception.

The SASCP contractors, Harry Bezuidenhout, Denzil Lawrie, H.J. Myburgh, Theo van Zyl for their enthusiastic work and support of the SASCP programme. Their assistance has brought about the physical control of *Sirex noctilio* in plantations by implementing the research guidelines.

My supervisor, Dr Terry Olckers, for his encouragement.

My co-supervisor, Dr Marnie Light, for all her assistance and support during the study period. I have learnt much from your patient editing and suggestions.

Dr Ilaria Germishuizen for all her assistance with the mapping for presentations, national monitoring point generation, *Sirex* climatic risk development and mapping application rollout to all the concerned landowners, and all the other queries we have run on the datasets.

The Institute for Commercial Forestry Research (ICFR) Management team for their acceptance of the SASCP into their structures, including their support and encouragement.

List of Abbreviations

DAFF	Department of Agriculture, Fisheries and Forestry
DWAF	Department of Water Affairs and Forestry
FABI	Forestry and Agriculture Biotechnology Institute
ICFR	Institute for Commercial Forestry Research
NMI	National monitoring initiative
PPRI	Plant Protection Research Institute
SASCP	South Africa <i>Sirex</i> Control Programme
TAPPSA	Technical Association of the Pulp and Paper Industry of South Africa
spHa	Stems per hectare

Terminology

Compartment	This is a stand of evenly-aged trees that are managed as a unit.
Stands	This is the same as a compartment.
Blocks	This a made up of many compartments as a consolidated area.
Plantation	This comprises several blocks.

Abstract

The invasive woodwasp, *Sirex noctilio* F. (Hymenoptera: Symphyta: Siricidae), a major global pest of pine plantations, was accidentally introduced into South Africa via Cape Town in 1994. *Sirex noctilio* initially spread along the southern coast of the Western Cape and into the Eastern Cape, remaining in the winter rainfall Mediterranean climate area. In 2004, *S. noctilio* crossed into the summer rainfall area of South Africa and by 2011 had reached the Limpopo Province. In KwaZulu-Natal, pine tree mortality averaged 5% per annum with some compartments reaching 20% over a 3- to 4-year period. During the initial spread of *S. noctilio* in the Western and Eastern Cape, *Ibalia leucospoides* Hochenwarth (Hymenoptera: Ibaliiidae) (a parasitic wasp) and *Deladenus siricidicola* Bedding (Nematoda: Neotylenchidae) (a nematode which renders *S. noctilio* eggs sterile), were introduced into these regions with success.

When *S. noctilio* entered the summer rainfall areas of South Africa, initial inoculations of *D. siricidicola* were unsuccessful. A mass inoculation campaign involving *D. siricidicola* followed in 2007 with subsequent inoculations taking place annually. It was evident after *S. noctilio* had entered the northern part of the Eastern Cape and KwaZulu-Natal that pine plantations in Mpumalanga and Limpopo were under threat. Therefore, the South African *Sirex* Control Programme (SASCP) was implemented to monitor the spread and impact of the pest.

As part of the SASCP, monitoring of *S. noctilio* and the two biological control agents over several years indicated an improvement in both *D. siricidicola* and *I. leucospoides* parasitism, which constrained the threat posed by the wasp. Population decreases of *S. noctilio* were verified at a regional level. Panel traps baited with kairomone lures also recorded the movement of *S. noctilio* through South Africa from KwaZulu-Natal, Mpumalanga and Limpopo Provinces. During this phase, a climatic risk mapping model for *S. noctilio* was completed and implemented, enhancing the placement of traps in high risk areas. The climatic risk mapping model was completed for South African forestry areas and distributed to the forestry owners, which allowed them to manage the *S. noctilio* risk on their properties. Once *S. noctilio* had migrated throughout South Africa, the National Monitoring Initiative (NMI) was implemented as a quantitative assessment of *S. noctilio* tree damage in permanent plots in pine-growing areas.

The outcomes of the SASCP that are compiled annually enabled the ranking of the different forestry regions in terms of their control status and shortcomings (e.g., low parasitism levels). Overall, the implementation of biological control and key aspects of monitoring both the pest and the biocontrol agents have been enhanced.

Key Words: Biological control, forestry pest monitoring, parasitic nematodes, risk assessments, wood-boring wasps.

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1.0. INTRODUCTION

1.1. Background to *Sirex noctilio* establishment in South Africa

Sirex noctilio F. (Hymenoptera: Symphyta: Siricidae) is an exotic wood-boring wasp that deposits the fungus *Amylosterium areolatum* (Chaillet ex Fr.) Boidin and a phytotoxic mucus during oviposition in various pine species of forestry value (Bedding, 1984; Tribe and Cillie, 2004). The combined effect of *A. areolatum* and the phytotoxic mucus is the desiccation of the affected trees to the extent that the dead timber is rejected by pulping and sawtimber mills, creating financial strain on the larger companies (du Plessis *et al.*, 2010). In 1994, *S. noctilio* entered South Africa through Cape Town (Tribe and Cillie, 2004) and by 2004, it was present in the northern parts of the Eastern Cape and KwaZulu-Natal. This caused high levels of mortality in pine plantations, with up to 30% mortality of trees older than 10 years and an annual increase in mortality of 5%, specifically in pulpwood compartments (P. Croft, personal observation). By 2011, *S. noctilio* was present in Limpopo Province having invaded all the pine-growing areas of South Africa.

Sirex noctilio is endemic to North Africa and Europe where natural control agents prevent such extensive population growth, thereby preventing severe tree mortality (Spradbery and Kirk, 1978). Biological control was thus considered the most promising control mechanism. The area under pine in KwaZulu-Natal is small compared to provinces further north in South Africa where plantations were vulnerable as *S. noctilio* moved northwards without any chemical, biological or cultural constraints. Research institutes required time to gain an understanding of the wasp and mechanisms causing tree mortality in order to make recommendations to curb tree mortality. Therefore, monitoring the populations and movement of *S. noctilio* during the research phase, and the subsequent implementation of biological control, was of utmost importance.

1.2. Impact of *Sirex noctilio* on the South Africa Forestry industry

In 1994, *S. noctilio* was reported as being established in the Cape Town region having entered through the city's port. The wasp subsequently invaded pine plantations in the Western Cape Province and moved towards George (Tribe and Cillie, 2004). At that time, the wasp was thought to be restricted to the winter rainfall region that supports a Mediterranean climate. *Sirex noctilio*, however, continued to move into the northern part of

the Eastern Cape and into KwaZulu-Natal Province, which constitute summer rainfall regions. Mortality of pine species in these regions was severe compared to the low mortality levels reported within the winter rainfall areas (Hurley *et al.*, 2007).

The high levels of tree mortality in the summer rainfall areas highlighted the fact that the invasion of *S. noctilio* in these areas was possibly due to ineffective releases of biological control agents, which were successful in the winter rainfall areas of the Western Cape Province. Thus, there was a need to improve both the effectiveness and deployment of the main biological control agent, *Deladenus siricidicola* Bedding (Nematoda: Neotylenchidae), to reduce the severe tree mortality, or to find alternative measures to control *S. noctilio*. Tree mortality due to *S. noctilio* reached 25% and increased at 5% per annum in many mature pulpwood compartments in the summer rainfall areas of KwaZulu-Natal (Hurley *et al.*, 2007).

To deal with this problem, members of the South African forestry industry exerted pressure on two research facilities, the Institute for Commercial Forestry Research (ICFR) and the Forestry and Agricultural Biotechnology Institute (FABI), as well as their own researchers, to research and implement control measures that would prevent further timber loss. The South African *Sirex* Control Programme (SASCP) was thus established in 2004 to steer and implement a co-ordinated, integrated pest management approach to control *S. noctilio* and prevent further tree mortality.

The South African forestry industry established a steering committee to formulate decisions to mitigate the *S. noctilio* problem. Some of these decisions included the involvement of government to fund pest control initiatives that affected all landowners, to avoid relying only on resources from larger forestry businesses. This included the building of a quarantine facility at FABI, University of Pretoria, to mass produce the nematode *D. siricidicola* (the main biological control agent of *S. noctilio*), in addition to other agents that were required to control invasive forestry pests in South Africa. Initially, when *S. noctilio* populations reached high levels in KwaZulu-Natal Province in 2007, no established facility in South Africa could produce the numbers of nematodes required to inoculate infested plantations. At that time, an Australian-based company assisted in producing the nematodes and exported them to South Africa for release in KwaZulu-Natal and the northern part of the Eastern Cape. However, the time taken to transport *D. siricidicola* over such long distances resulted in up to 50% mortality of the nematodes, due to their short life span (up to a week). FABI have since utilised this quarantine facility to produce enough *D. siricidicola* to satisfy the demand of the

forestry industry and enable the SASCP to facilitate the control of *S. noctilio* in plantation forestry in South Africa.

The SASCP's united effort has suppressed populations of *S. noctilio* to varying levels across South Africa. This was facilitated by funding from the Department of Agriculture, Forestry and Fisheries (DAFF) for operational expenses, the involvement of Forestry South Africa (FSA) in supporting the industry SASCP Co-ordinator, as well as research organisations including FABI and ICFR. All forestry companies and private growers in South Africa have participated in the SASCP, allowing the release of biological control agents. When established in 2004, the main research focus areas of the SASCP were to understand the biology of *S. noctilio* in South Africa, locate biological control agents that were effective in other parts of the world, and to test their compatibility for controlling *S. noctilio* under South African climatic conditions. These control agents needed to be tested prior to release in South Africa, to predict their natural distribution and effectiveness in South Africa.

Sirex noctilio has had a major impact on the South African forestry industry, affecting both large and small growers. In 2007, several methods were employed to quantify the impact of *S. noctilio* in South Africa and included the: area affected by *S. noctilio*; costs associated with additional silvicultural practices because of infestations; financial losses resulting from reduced timber sales; losses in value-added timber processing and; costs associated with the deployment and rearing of biological control agents. Losses caused by *S. noctilio* were determined by the Technical Association of the Pulp and Paper Industry of South Africa (TAPPSA) in 2008, based on 2006 forestry data (**Table 1.1**).

Table 1.1. Volume and loss of income associated with *Sirex noctilio* damage as estimated by TAPPSA in 2007, based on 2006 data (R. Godsmark, pers. comm.)

Description	Growers*	Processors*
Current best case scenario (current in 2007)		
Area affected	91 000 ha	
Inoculation cost	R 20 million	
Thinnings – operational cost	R 14 million	
Thinnings – value of volume lost	R 112 million	R 474 million
Volume output lost	578 000 m ³	198 000 m ³
Value of output lost	R 153 million	R 630 million
Future worst case scenario		
Area affected	279 000 ha	
Inoculation cost	R 73 million	
Thinnings – operational cost	R 103 million	
Thinnings – value of volume lost	R 724 million	R 3 157 million
Volume output lost	1 417 000 m ³	501 000 m ³
Value of output lost	R 383 million	R 1 527 million
Total potential costs		
Minimum	R 299 million	R 1 104 million
Maximum	R 1 283 million	R 4 684 million

* Growers refer to the plantation owners who grow the timber and sell it to the processing plants. Processors utilize the raw timber and produce the final products.

Quantifying the area impacted by *S. noctilio* revealed that for *Pinus patula* Schiede ex Schltl. & Cham. pulpwood compartments older than 10 years in KwaZulu–Natal lost an average 5% of their mature trees. Between 2006 and 2008, tree losses increased to between 15-20% cumulatively because of high wasp infestation levels during that period. The resulting dead timber could not be utilised profitably in any pulp processing plants since it had a negative impact on the paper making process, resulting in poorer quality paper (du Plessis *et al.*, 2010). Manufacturing paper from dehydrated fibres / *Sirex*-infested and young trees produced paper with consistently lower strength properties compared to wood obtained from healthy trees and trees over 12 years of age. The thermo-mechanical process required higher energy consumption to produce pulp from *Sirex*-infested logs while the end product

was inferior, having a negative lower burst, tear and breaking length paper quality (du Plessis *et al.*, 2010).

Infestations by *S. noctilio*, in addition to killing trees, increased silviculture costs. In severe situations, especially in 2007 and 2008, harvesting was implemented as a control method. This resulted in large quantities of brushwood (predominately dead timber from *S. noctilio* activity) being stacked and burnt to provide access for planting teams. Bulldozers were required to move the brushwood into stockpiles for burning. The costs associated with the use of such equipment and the associated labour increased the costs to far above that of the normal silvicultural practices.

A reduction in saleable tonnage from tree losses led to landowners seeking alternative markets in an attempt to offset financial losses. The impact of *S. noctilio* on timber growers was extended to losses in the value-added benefits associated with raw material products. This was highlighted in a model, constructed by the FSA Operations Director, Roger Godsmark, who predicted the loss in volume and income from *S. noctilio* in South Africa. Predicted losses for growers ranged from R 3 million to R 1 283 million while those for processors exceeded these levels (**Table 1.1**).

To mitigate the impact of *S. noctilio*, biological control was implemented in 2007 to reduce the wasp's population density, thereby reducing the loss of timber. Costs associated with using the primary biological control agent *D. siricidicola*, imported from Australia, were calculated at R 52.00 per tree. In addition, costs associated with the deployment of contractors to search for and inoculate infected trees were approximately R 50.00 per tree. Thus, in 2007, 80 000 trees were planned for inoculation at an expected cost of R 8 160 000 (for both the *D. siricidicola* and labour costs).

1.2.1. Plantation tree losses in South Africa

The determination of tree losses required accurate assessments of individual trees, which were attacked by *S. noctilio*. The criteria that enabled recognition of infested trees included:

- *Sirex noctilio* eggs and larvae in larval galleries that contain fine frass in the areas through which the larvae have passed,
- The presence of oviposition drills that are marked with resin droplets on the bark exterior,

- Dead larvae or female wasps suspended by their ovipositor on the bark,
- Trees where the wasp has completed its life cycle, as indicated by visible round adult exit holes. These holes are visible and proceed through the bark and into the timber of the tree before turning to follow the timber grains (Coutts, 1965),
- The presence of oviposition sites which cause long narrow pale brown streaks in the direction of the grain, above and below the oviposition site. This wood is drier than the surrounding wood. These streaks are overlaid by a brown stain which can be seen on the underside of the bark (Coutts, 1965),
- The discolouration of the crowns where the crown turned brown as a result of the mucus injected by the female wasp during oviposition (Coutts, 1969a).

The extent of tree losses needs to be considered in relation to the susceptibility of the pine species that were planted within the various geographical regions of South Africa at the time of *S. noctilio* attack. Hinze (1993) reported that in 1991 softwoods occupied 50% of the total area under commercial plantations. Of the 648 568 ha of softwood plantations as at 31 March 1991, 290 943 ha (45%) comprised *P. patula*, 178 653 ha (28%) *P. elliottii* Engelm., 65 541 ha (10%) *P. taeda* L., 56 154 ha (9%) *P. radiata* D. Don, 37 970 ha (6%) *P. pinaster* Aiton and 19 307 ha (3%) other species (Hinze, 1993). The change in plantation species composition between 1991 and 2012 (**Figure 1.1**) shows a decrease in *P. pinaster* and *P. taeda* with an increase in *P. patula*. This had a positive impact on *S. noctilio* populations due to the 45% dominance of *P. patula*, which is the preferred host species.

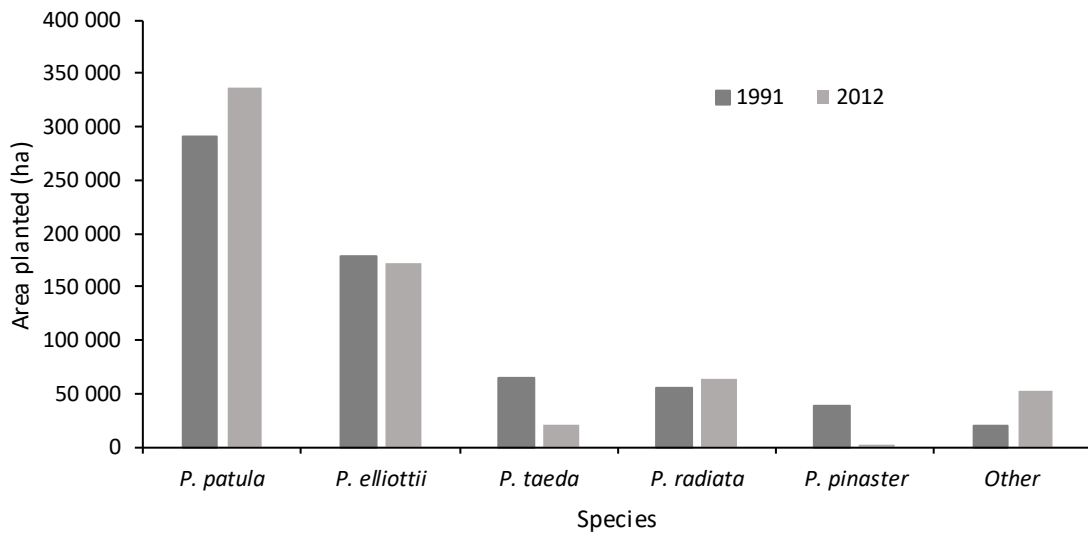


Figure 1.1. South African pine plantings as at 1991 and 2012 indicating the change in plantation species composition.

Table 1.2 shows the distribution of *Pinus* species across South Africa and illustrates the fact that *S. noctilio* had a suitable pine plantation habitat, from Cape Town in the Western Cape Province through semi-contiguous timber producing Provinces to Limpopo Province, except for Zululand and Maputoland where higher temperatures prevented *S. noctilio* from becoming established. Plantations for pulpwood production were planted with *P. patula*, while sawtimber plantations were planted with *P. patula*, and to a lesser extent to *P. elliottii* and *P. taeda*.

Table 1.2. Pine species distribution by region in 2012 (Meyer, 2012).

Regional Pine plantings 2012	Softwood		Species breakdown						Total
	Sawtimber	Pulpwood	<i>P.patula</i>	<i>P.elliottii</i>	<i>P.taeda</i>	<i>P.radiata</i>	<i>p.pinaster</i>	Other	
Northern Regions									
Limpopo Province	26 887	56	15 711	6 114	921	53	0	4 180	26 979
Mpumalanga Province	134 283	28 707	73 275	59 270	10 598	0	0	19 847	162 990
Central Districts	3 518	15 983	15 765	1 748	4	0	0	1 988	19 505
Mpumalanga South	64 872	57 889	86 207	30 301	2 157	0	0	6 413	125 078
Total Northern Regions	229 560	102 635	190 958	97 433	13 680	53	0	32 428	334 552
Middle Regions									
Maputuland	0	8 225		7 365					7 365
Zululand	3 715	283	1 525	2 064	280			129	3 998
Northern KwaZulu-Natal	9 011	8 649	5 482	10 753	300	0	0	1 126	17 661
KwaZulu-Natal midlands	26 434	37 475	37 849	20 219	2 441	0	0	3 402	63 911
Southern KwaZulu-Natal	28 141	18 216	33 811	9 475	806	609	0	1 656	46 357
Total Middle Regions	67 301	72 848	78 667	49 876	3 827	609	0	6 313	139 292
Southern Regions									
Eastern Cape	85 731	14 612	49 359	24 335	3 090	11 325	31	12 203	100 343
Southern Cape	56 845		18 278	1 305	0	37 309	49	11	56 952
Western Cape	14 753		207	56	115	14 144	219	20	14 761
Total Southern Regions	157 329	14 612	67 844	25 696	3 205	62 778	299	12 234	172 056
Total South Africa	454 190	190 095	337 469	173 005	20 712	63 440	299	50 975	645 900
	644 285								

Of all the regional pine plantings in South Africa in 2012 (**Table 1.1**), *P. radiata*, *P. elliottii* and *P. patula* were planted most extensively, with *P. patula* and *P. radiata* being the most susceptible to *S. noctilio*.

The survival of pine trees, especially in pulpwood compartments, which consisted of higher stocking with no thinning regimes, in the northern part of the Eastern Cape and KwaZulu-Natal Provinces, were substantially impacted by *S. noctilio* from 2004 to 2007. Tree mortality up to 5% per annum was experienced, resulting in cumulative tree losses of 20% - 25% in severely affected areas during this period (**Chapter 5**).

Tree loss can be increased by short periods of physiological stress experienced by individual trees (Madden, 1988). This stress appears to be sufficient to stimulate the increased diffusion of carbon dioxide from the stem, which together with the phloem having a greater degree of enzymatic oxidation, increases the tree's attractiveness to *S. noctilio* and permits successful oviposition (Madden, 1968a; 1968b). The stimulation of stress signals within the tree can be brought about by damage from wind or harvesting operations, defoliation, herbicides, fire, cone harvesting and thinning, especially if these operations are carried out during the wasp's flight season (Madden, 1988).

1.3. Background information to the South African *Sirex* Control Programme

1.3.1. Introduction to the South African *Sirex* Control Programme

The development and success of the ongoing SASCP relied heavily on field activities, which monitored the populations of *S. noctilio* across South Africa. These field operations were expensive and funding was required to maintain trained, properly equipped and dedicated field contractors who were capable of managing operations at the required operational standard. Field operations collected the data, which allowed the SASCP to manage the process.

1.3.2. Resources for the South African *Sirex* Control Programme

1.3.2.1 Funding

Funding by the then South African Department of Water Affairs and Forestry (DWAFF, now DAFF) which was responsible for Timber Plantation management, enabled the necessary field operations and distribution of high numbers of healthy nematodes across South Africa, which ultimately led to the success of the SASCP in controlling *S. noctilio*.

1.3.2.2. Organisational structure

Four key components of the SASCP have enabled the various role players to reach their full potential within the SASCP and remain focussed on reducing the threat of *S. noctilio*. These key components include:

- Biological control (research and commercial initiatives);
- Monitoring;
- Awareness; and
- Research to develop a knowledge base on *S. noctilio*.

This thesis emphasises the monitoring aspect of the SASCP.

1.3.2.3. Complexities of Forestry in South Africa

South Africa, unlike other countries, has numerous landowners producing a multitude of timber products, with more small growers being empowered as larger corporate entities sell off forestry land. Ensuring that all owners were aware of, and became involved in, the SASCP made the initiative more challenging.

In South Africa, forestry occupies 1.1% or 1 268 443 ha of the available 119 300 000 ha. Forestry land is either privately (83.3%) or publicly (16.7%) owned, and is subdivided between softwoods and hardwoods. Softwoods occupy 646 758 ha or 50.9% of forestry land and are used for the production of sawtimber and veneer logs (56.2%), pulpwood (42.2%), and poles and other products (1.6%). The hectares under cultivation in each of the provinces

producing softwood products from various species of *Pinus* (species indicated in **Table 1.2**) in South Africa, are indicated in **Table 1.3** (DAFF., 2014).

Table 1.3. Provinces that produce softwood in South Africa, indicating the hectares under cultivation and percentage contribution to the entire forestry area (DAFF., 2014).

Province	Hectares	Percentage of Total	Rainfall region
Limpopo	26 979	4.1%	Summer
Mpumalanga	307 573	47.6%	Summer
KwaZulu-Natal	140 151	21.7%	Summer
Eastern Cape	118 653	18.3%	Winter, All year, Summer
Western Cape	53 402	8.3%	Winter
	646 758	100%	

Sirex noctilio initially entered the Western Cape Province in South Africa, which affected only 8.3% of the total forestry area. Once the wasp had become established in the Western Cape Province and had spread to the Eastern Cape Province, 73.4% of the softwood forestry area became vulnerable, especially since *S. noctilio* could adapt to a summer rainfall climate.

1.3.2.4. South African Provinces and regions

South Africa comprises nine provinces (**Figure 1.2**) with five provinces utilized, to various proportions, for growing pine species, based on climate, soil and proximity to markets. A Forest Economic Services report compiled for DAFF (Meyer, 2008) divided the country into 12 regions (**Figure 1.3**) and reported on the planted hectares and volume of timber grown for various markets against these 12 regions. The national monitoring initiative used this report to allocate semi-permanent monitoring plots in a randomised design across these 12 regions.

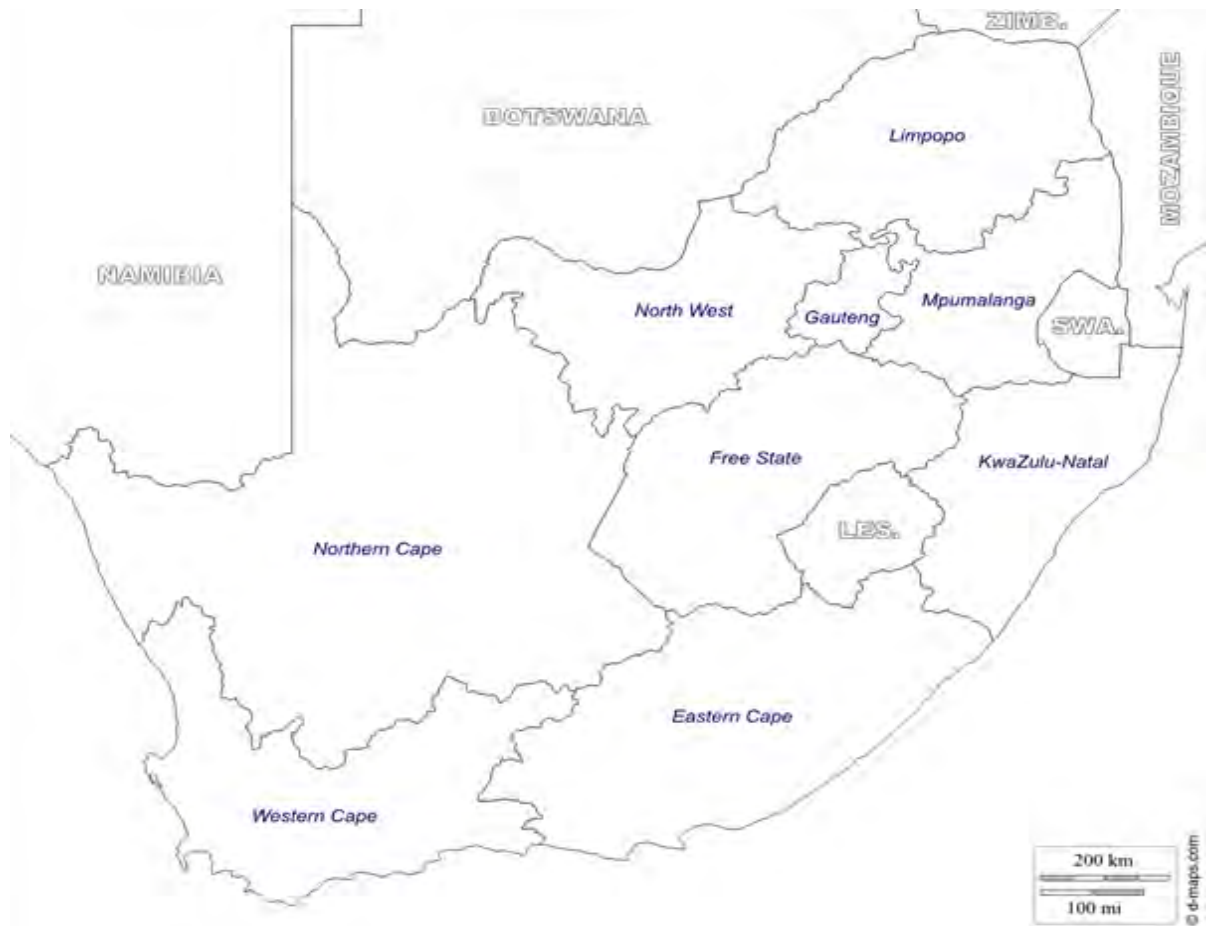


Figure 1.2. The nine South African provinces. From: http://www.d-maps.com/carte.php?num_car=4415&lang=en)

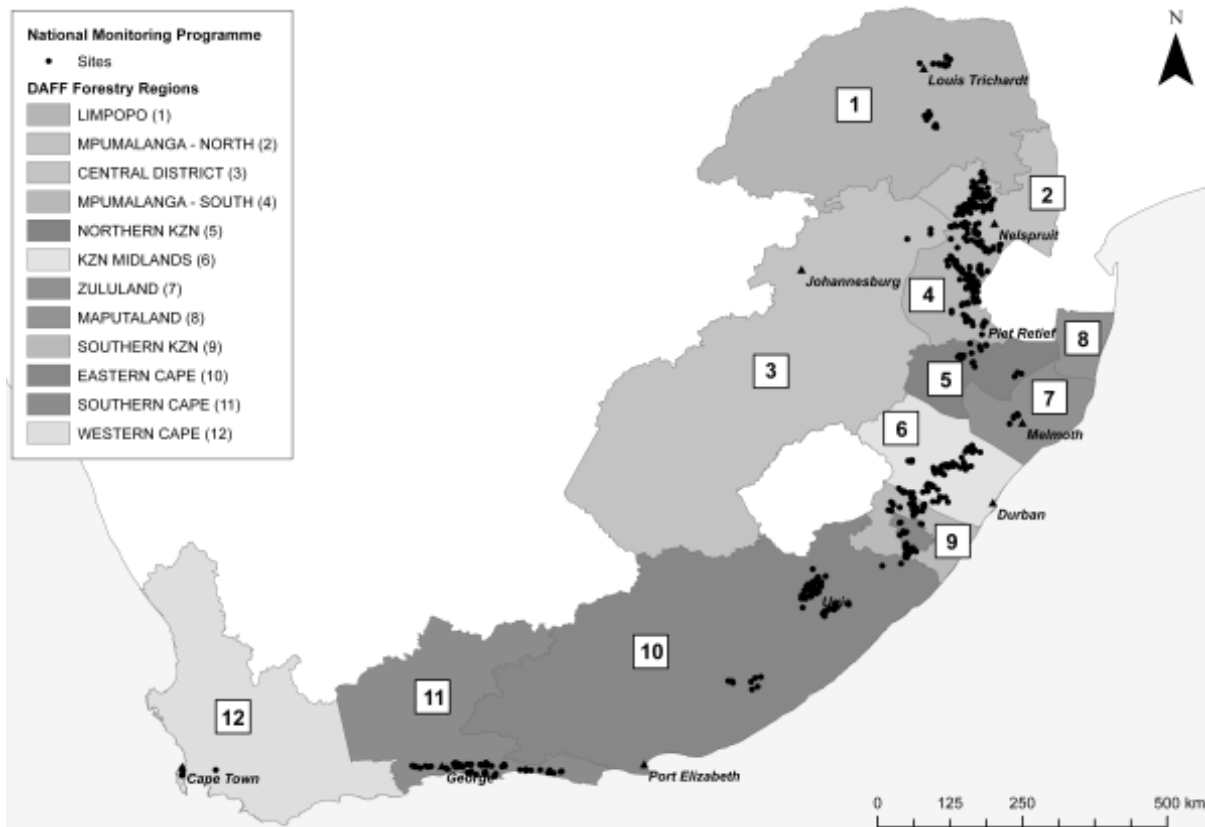


Figure 1.3. The 12 DAFF regions as defined in the Forest Economic Services report (Meyer, 2008) and used for the selection of monitoring sites for this study

1.4. Thesis aims and objectives

The main objectives of this study were:

- To record the movement of *S. noctilio* within South Africa (**Chapter 4**) as the pest invaded different pine-growing areas, to ensure that biological control was implemented timeously and monitored for successful establishment of the agents.
- To record the levels of parasitism that were achieved by the two biological control agents, *Deladenus siricidicola* and *Ibalia leucospoides* (**Chapter 3**), to gain an understanding of their levels of success in controlling *S. noctilio* in South Africa's diverse rainfall regions.
- To develop and report on the National Monitoring Initiative (**Chapter 5**) through the placement of monitoring plots determined by the *S. noctilio* bioclimatic risk rating model (**Chapter 6**), to illustrate national trends of biological control success.

1.5. Thesis structure

The involvement of the SASCP co-ordinator in managing the various monitoring aspects led to the accumulation of records, which are expanded on in various thesis chapters.

In **Chapter 2**, a literature review details the aspects relevant to the monitoring of *S. noctilio* damage and population densities in South Africa.

In **Chapter 3**, parasitism of *S. noctilio* was determined by dissecting wasps sampled from sample logs to determine the effectiveness of the nematode inoculations and the background parasitism. Three depots with over 1200 cages were utilised annually to determine parasitism across the various timber-growing regions of South Africa.

Chapter 4 reports on the movement of *S. noctilio* throughout South Africa as determined by the use of panel traps baited with a kairomone lure.

Chapter 5 reports on the termination of the panel trap monitoring and the development of a national monitoring initiative, whereby semi-permanent plots were laid out across the South African forestry regions to determine the levels of tree damage caused by *S. noctilio*.

Chapter 6 deals with the development of a climatic risk-rating model, its implementation across the forestry areas of South Africa, and its impact in facilitating improved *S. noctilio* management.

The status of *S. noctilio* in South Africa in 2014 is discussed in **Chapter 7**, in the context of the success attained by the SASCP and the areas that need further attention.

2.0. LITERATURE REVIEW

This literature review provides the necessary background information regarding monitoring and integrated pest management approaches for *S. noctilio* and does not cover all the in-depth aspects of the wasp's life cycle.

2.1. Woodwasps

Woodwasps or horntails (Hymenoptera: Siricidae) are large, colourful stingless wasps whose larvae bore into tree stems (Sharkey, 2007). They are native to North America, Eurasia and North Africa (Hurley *et al.*, 2007; Bordeaux and Dean, 2012), feeding on coniferous and hardwood trees (Madden, 1988) and causing economic damage to marketable timber (Madden, 1988; Hurley *et al.*, 2007). Trees that tend to be affected are mainly suppressed trees such as those that are un-thinned or drought stressed (Neumann and Minko, 1981). The most notorious species in this family is *Sirex noctilio* Fabricius, which utilises a fungus, *Amylosterium areolatum* (Chaillet ex Fr.) Boidin, and a toxic mucus to control the moisture content of infested pine trees. This creates optimal moisture conditions for growth of the fungus, which is the food source for the *S. noctilio* larvae. The damage caused to the pine trees is the combined effect of the fungus that severely reduces the moisture content of the wood, the phytotoxic mucus that breaks down the needles, and the larvae that create tunnels or galleries deep into the tree (Tribe and Cillie, 2004). During oviposition, *A. areolatum* is deposited into the tree and as the fungus grows, it allows the larvae to move through the softened wood (Spradbery and Kirk, 1978). The phytotoxic mucus, which is also deposited during oviposition, is translocated to the pine needles where it breaks them down, preventing photosynthesis and disrupting moisture evaporation (Coutts, 1969a).

2.1.1. *Sirex noctilio*

2.1.1.1. International migration of *Sirex noctilio*

Sirex noctilio is native to Eurasia and North Africa (Fenili *et al.*, 2000) but has spread across many pine-growing countries. The wasp was discovered in New Zealand in about 1900, Tasmania in 1951, the Australian mainland in 1962, Uruguay in 1980, Argentina in 1985, and

Brazil in 1988 (Hurley *et al.*, 2007). In 1994, *S. noctilio* was first discovered in South Africa (Tribe, 1995) with more recent discoveries in Chile in 2001 (Beeche *et al.*, 2012) and in North America in 2004 (Hoebeke *et al.*, 2005).

2.1.1.2. Host trees for *Sirex noctilio*

Sirex noctilio has been reported from several genera of trees including *Pinus*, *Abies*, *Larix*, *Picea*, *Pseudotsuga* and *Araucaria*, with *Pinus* species considered to be the preferred hosts (Madden, 1988) .

Pinus radiata is the dominant host species in Australia, New Zealand, Chile and the winter rainfall region (Western Cape Province) of South Africa (Ryan and Hurley, 2012). In the wasp's natural range, *P. pinaster* was recorded as the most attacked species (Spradbery and Kirk, 1978). In Brazil, *P. taeda* and *P. elliottii* are preferentially attacked, whereas *P. elliottii*, *P. ponderosa*, and *P. radiata* are attacked in Argentina and Uruguay. In the summer rainfall areas of South Africa (northern Eastern Cape, KwaZulu-Natal, Mpumalanga and Limpopo Provinces), *P. patula* has been the most infested species (Ryan and Hurley, 2012), as well as the most extensively planted pine species. In comparison, the dominant tree species attacked in the Northern Hemisphere, specifically in the USA and Canada where *S. noctilio* was recently accidentally introduced, include *P. banksiana*, *P. sylvestris*, *P. resinosa* and *P. strobus* (Dodds and de Groot, 2012). Other species across the world include *P. pinea*, *P. echinata*, *P. halepensis*, *P. palustris*, *P. brunia*, *P. nigra*, *P. contorta*, *P. jefferyi*, *P. chaipensis* and *P. greggii* (Spradbery and Kirk, 1978; Tribe, 1995; Iede *et al.*, 1998; Maderni, 1998; Hoebeke *et al.*, 2005). No species of *Pinus* has demonstrated resistance to attack, although there are varying levels of susceptibility to *S. noctilio* (Maderni, 1998).

2.2. *Sirex noctilio* in South Africa

2.2.1. Movement in South Africa

In South Africa, *S. noctilio* was initially recorded in plantations of *P. radiata* in Tokai, Cape Town in 1994 (Tribe and Cillie, 2004). *Sirex noctilio* has since moved northwards, causing severe losses to pine timber, especially to *P. patula* that was grown for pulpwood in the

northern part of the Eastern Cape and KwaZulu-Natal Provinces. Since 2011, the northern most distribution of *S. noctilio* was confirmed as the Limpopo Province of South Africa (**Chapter 4**).

2.3. Life cycle of *Sirex noctilio*

2.3.1. Biology of *Sirex noctilio*

Most of the life cycle of *S. noctilio* is spent as a larva in the sapwood of the host tree, which makes it difficult to detect. The larval growth and reproductive potential of *S. noctilio* is linked to the vigour of the basidiomycete fungal symbiont, *A. areolatum* (Coutts and Dolezal, 1965). The interaction between *S. noctilio*, *A. areolatum* and the phytotoxin allows the wasp to breach the defences of its living host tree.

The eggs of *S. noctilio* are white, soft and smooth (Zondag and Nuttal, 1977) and 1 mm in length (Coutts, 1965). Larvae are cylindrical and creamy yellow with a round head (**Figure 2.1.E**). They have short legs and a black tail spike. Female larvae have “hypopleural” sacs, which are difficult to see. Sex determination is facilitated by looking on the underside of the ninth abdominal segment where females have two small hardened brown zones as opposed to three in male larvae (Zondag and Nuttal, 1977).

The *S. noctilio* wasps have four membranous wings and a stout cylindrical body, which tapers to a point at the rear. Adult size is variable, ranging from 9 to 36 mm from the front of the head to the tip of the abdomen (Zondag and Nuttal, 1977). The female *S. noctilio* wasps (**Figure 2.1.A**) have metallic dark blue bodies with amber coloured wings and legs and black tarsi. The protective sheath containing the ovipositor projects beyond the abdomen by 2 to 3 mm. The male wasps (**Figure 2.1.B**) are metallic dark blue, except for their easily identifiable abdominal segments, wings, front and mid legs that are chestnut brown. The tail end of the male abdomen is metallic dark blue (Neumann and Minko, 1981). The antennae of both sexes are black.

Emerging *S. noctilio* adults have fully developed reproductive systems with female wasps able to start laying eggs on the day of emergence. Female wasps live for three to four days, laying 50 to 500 eggs, depending on body size. Both adult male and female wasps do not feed, but live off stored body fat, allowing them to live for approximately 12 days, although

active egg laying females will only live for three to four days (Zondag and Nuttal, 1977). Female wasps seek the sapwood of physiologically stressed coniferous and hardwood trees in which they lay eggs during the flight season, whereas males remain in the tree canopy where mating occurs (Madden, 1974; Villacide and Corley, 2008; Wermelinger and Thomsen, 2012). Prior to egg laying, the female assesses the suitability of a host tree by walking over the bark and probing the phloem with her ovipositor. Acceptance or rejection of the tree takes place after two or three probes.

Host suitability is assessed by sensilla in the female's ovipositor that determine the moisture content of the tree and resin pressure of the wood prior to oviposition. If the moisture content is between 30% and 80%, the female will proceed and lay eggs but not outside this range (Coutts, 1965). Within a tree, drought stress increases the concentrations of several individual resin acids and reduces growth parameters (Tortola *et al.*, 2003), which makes a tree more attractive to female *S. noctilio* wasps. When the tree phloem sap is under high osmotic pressure (>12 atm), the tree is rejected for oviposition (Madden, 1974).

After copulation, the females store sperm in their spermathecae. During oviposition, as the egg passes down the oviduct, the female may or may not release sperm to fertilize the egg. If no sperm are released the offspring are males, but if sperm is released then the offspring are either male or female (Sharkey, 2007). Some female wasps may oviposit before mating, also resulting in male offspring (Stillwell, 1966).

General oviposition behaviour is such that the female *S. noctilio* will either face up the tree or down towards the ground. The ovipositor is then extended from the sheath in search of crevices in the tree bark. The ovipositor penetrates the bark (**Figure 2.1.A**) and wood, either partially or completely. After an egg is deposited, the ovipositor is rapidly withdrawn, either completely or partially. When completely withdrawn, a single egg is deposited at that site. When partially withdrawn, with the tip remaining in the bark, the female tilts her body and deposits a second egg into a different location in the wood, ensuring that the eggs are separated in the cambium layer. One to three oviposition tunnels are most common, but four or five tunnels have been observed. If the ovipositor meets hard late-wood, it makes a sharp curve depositing the egg at the end of the tunnel (Coutts, 1965). Single oviposition tunnels are drilled but not all may contain eggs. When multiple tunnels are drilled, these all contain one and sometimes two eggs, but the last tunnel is not utilized for oviposition but for the deposition of the fungus *A. amylosterium*.

When the tree has very high or very low moisture content, female wasps drill only one hole. At intermediate moisture content, the ratio of single holes is decreased, the number of triple holes is increased, with the number of double holes being more consistent through the various moisture content readings (Coutts, 1965).

The oviposition tunnels are between 5 to 9 mm in length and 0.5 mm in diameter (Coutts and Dolezal, 1965; Zondag and Nuttal, 1977), and are determined by the wood characteristics.

There are three scenarios in relation to this (Coutts, 1965; Madden, 1974):

- Should the annual rings be wide enough for the ovipositor to penetrate the wood without penetrating the annual ring, then these long oviposition tunnels may have two eggs laid in them.
- If the outer ring of summer wood is 6 mm wide, and the ovipositor can penetrate the annual ring, then the female wasp will drill into the next summer wood area, with one or more eggs being laid.
- Should the outer growth rings be narrow and difficult to penetrate, then a short tunnel is drilled with one egg being deposited.

The 1 mm long egg is placed towards the end of the oviposition tunnel. If a second egg is laid, it is placed slightly away from the first egg, or even towards the entrance of the oviposition tunnel where it is visible if the bark is removed.

The eggs of *S. noctilio* take about 9 days to hatch (Zondag, 1959). When the larvae are about to hatch, hyphal strands of *A. areolatum* have already created a network across the oviposition tunnel. The hatching first-instar larvae start to tunnel, creating the larval cavities or tunnels, either upwards or downwards in the tree. Eggs situated in very dry timber, with a moisture content of 20% to 26%, fail to hatch (Coutts, 1965).

Below the bark, above the oviposition hole, a dark stained necrotic lesion becomes visible. This elongated lens-shaped wood stain, reddish brown in colour, increases in size to cover the bole of the tree. This response by the tree occurs as a result of either larval wounding, *A. areolatum* or the mucus deposited by the wasp (Eldridge and Taylor, 1989).

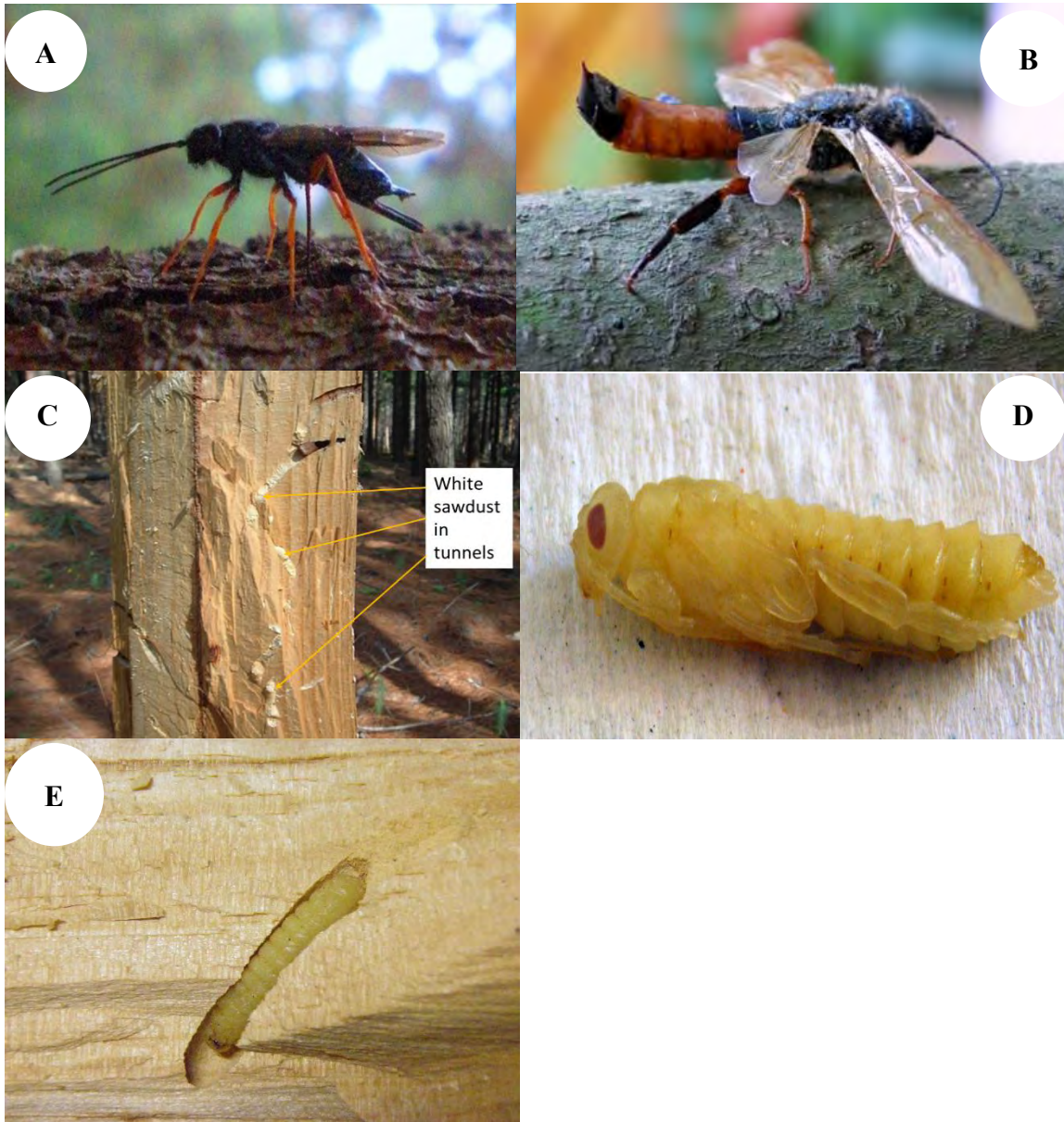


Figure 2.1.A. *Sirex noctilio* female ovipositing. Female seen with the ovipositor penetrating the bark and timber.

Figure 2.1.B. *Sirex noctilio* male. The orange band on the abdomen makes the male easy to distinguish from the female.

Figure 2.1.C. *Sirex noctilio* larval tunnels closed up with white sawdust. This tree was struck by lightning and split open, revealing the larval tunnels reaching the mid stem region.

Figure 2.1.D. *Sirex noctilio* pupa showing parts of the larva and developing wasp.

Figure 2.1.E. *Sirex noctilio* larva in a tunnel in the tree.

Delayed hatching may occur when temperatures within the tree remain low for most of the year (Madden, 1974). The first-instar larvae hatch and feed off the egg yolk and fungal mycelia within the oviposition hole (Madden, 1988). Larvae from the second instar feed on the fungus-infested timber, creating galleries that increase in diameter as the larvae grow. The larval tunnels or galleries are generally in the direction of the timber grain and as these are formed (**Figure 2.1.C**), the lightly coloured frass or sawdust is forced into the tunnel behind the larvae (Ayres *et al.*, 2009). The larvae make a U-turn when they enter the less nutritious heartwood and return towards the cambium (Tribe and Cillie, 2004). The number of larval instars varies from 6 - 12 (minimum of 3) depending on the condition of the timber. Factors increasing the number of larval instar moults are high moisture content resulting in poor fungal growth, physical barriers like resin pockets and changes in tunnelling direction (Madden, 1988). In the summer rainfall areas of South Africa, the larvae are visible in the timber from mid-February and increase in size until October and November when pupation takes place.

Pupation (**Figure 2.1.D**) takes place just under the bark (Tribe and Cillie, 2004) and is triggered by increased air temperature and simultaneous moisture content increase, which prevents the development of fungal growth (Madden, 1988). Following pupation, the male offspring emerge first with the females following shortly thereafter (Spradbery and Kirk, 1978). Both males and females display a strong photopositive response and fly upwards into the tree crown. Males swarm just above the tree tops. Mating takes place in the upper canopy, after which the females leave in search of suitable oviposition sites (Madden, 1988; Crook *et al.*, 2008). A short-range cue is required for mating to occur, as both sexes congregate in the upper branches of trees that are in the vicinity of trees from which adults have emerged (Crook *et al.*, 2012).

Adult emergence patterns have been studied in the wasp's native regions by using logs containing *S. noctilio* eggs and larvae (Spradbery and Kirk, 1978). The identical male-female emergence trend has been observed in South Africa (**Chapter 3**) and revealed that *S. noctilio* emergence started as early as June and July and lasted for approximately three months, with the last emergence ending in October. However, in Belgium emergence ended in January (Spradbery and Kirk, 1978).

In Australia, adults emerged from mid-November to April, with peak emergence during March (Tribe and Cillie, 2004). In Brazil and South Africa's summer rainfall area, emergence is from October to December (Iede *et al.*, 1998). In South Africa's summer

rainfall areas, which include the northern part of the Eastern Cape, KwaZulu-Natal, Mpumalanga and Limpopo Provinces, the emergence period lasts from the end of October to mid-December, with wasps in the Eastern Cape and KwaZulu-Natal emerging one week earlier than those in Mpumalanga and Limpopo. A completely different pattern of emergence is present in the winter rainfall areas of South Africa, namely the Western Cape Province, where emergence starts in late November and lasts until the first week of May. Under these conditions, there are no peak emergence periods, but rather a fluctuation in emergence depending on temperature and rainfall, with more *S. noctilio* emerging on warmer days and fewer on colder wet days (P. Croft, personal observation).

2.3.2. *Amylostereum areolatum* interaction with *Sirex noctilio*

Sirex noctilio has a symbiotic relationship with the fungus *A. areolatum* (Hurley *et al.*, 2007; Crook *et al.*, 2008), which is injected into the tree during oviposition and is required for the survival of *S. noctilio* larvae (Coutts, 1969b; a; Spradbery, 1973). The genus *Amylostereum* (Fries) Boidin was established in 1958 (Slippers *et al.*, 2002; Slippers *et al.*, 2003) and *A. areolatum*, in its invaded environments, reproduces asexually and is spread by female *S. noctilio* wasps. Only female wasps transport the spores of *A. areolatum*. *Sirex noctilio* larvae collect the fungus as arthrospores, which are stored in the hypopleural sacs found in the abdominal cavity. After each larval moult, these hypopleural sacs are re-contaminated with the fungal arthrospores. During pupation, the arthrospores are protected within mycangial sacs located at the base of the female's ovipositor (Francke-Grosman, 1939; Madden, 1988).

The fungus has a drying effect on the host wood causing white rot. The fungus provides a source of nutrients for the *S. noctilio* larvae. Within a year of *A. areolatum* infection, secondary decaying fungi enter through the *S. noctilio* exit holes causing accelerated timber decay (Fenili *et al.*, 2000). The drying of *S. noctilio*-infested wood is not uniform throughout the tree. The fungus is deposited at the bottom of the oviposition tunnel and grows into the wood and then towards the cambium. This process also follows the direction of the wood grain and forms typical dark brown streaks above and below the oviposition site, which indicates drier wood infested with *A. areolatum*.

The drying process spreads slowly tangentially and slower radially, and is retarded by the late wood annual rings. The impact of *A. areolatum* on the wood is such that the rate of drying is

rapid in the summer months after oviposition and gradually slows down in the drier winter months, as the tree dries out (Coutts, 1965). Initially, the fresh tip of the tree is wetter than the butt end of the tree, but as the process continues, the tip becomes drier than the butt end. The middle section of the tree dries at a rate in-between that of the tip and butt end. When a tree is felled in preparation for nematode inoculation, and left lying on the ground, the upper side initially dries more quickly than the underside, until the tree reaches 50% moisture content when the upper and lower portions become more consistent in moisture content. The outer centimetre of wood is generally wetter than the inner portion of wood, until the 50% moisture content level is reached when both portions are equal in moisture content (Coutts, 1965).

In the winter period, when the larvae are growing and developing through their various instars, the moisture content fluctuates between 30% and 50% and at wasp emergence the moisture content of the timber is around 20% (Coutts, 1965).

2.3.3. The impact of the mucus associated with *Sirex noctilio*

During the egg laying process, the female wasps inject a mucus into the oviposition tunnels. The mucus enters the sap stream of the tree and is translocated to the needles. It displaces some of the moisture and retards the translocation of moisture, thereby reinforcing the moisture stress of the tree (Fong and Crowden, 1973). The mucus causes premature senescence by reducing the chlorophyll content in the needles, and increasing respiratory activity of the tree by up to 2.5 times the normal daily rate. These symptoms occur rapidly within affected trees, indicating rapid fungal translocation to the needles. This results in some impairment of the normal water relationships within the needles causing desiccation, distortion, and eventual collapse of the translocatory phloem cells (Fong and Crowden, 1973).

The mucus was characterised by Boros in 1968 as a protein-mucopolysaccharide complex (Coutts, 1969a). The enzyme activity of the mucus is active on the physiological processes of the tree. Under research conditions, pine trees inoculated with mucus changed colour after 12 days with more starch present in the needles than in trees that were irrigated and inoculated with mucus, which produced no colour change and little trace of starch in the needles (Coutts, 1969a).

A study by Boros (1968 in Coutts, 1969a) showed that when *S. noctilio* mucus was added to water agar with *A. areolatum*, it stimulated the growth of *A. areolatum* (Coutts, 1969a). Further studies have shown that the mucus prolongs the predisposing tree stress condition by preventing the translocation of sugars (Madden, 1988; Bedding and Iede, 2005). This allows the development of *A. areolatum* mycelia, which in turn block the movement of nutrients and water through the xylem of the tree (Madden, 1988).

2.3.4. *Amylosterium areolatum* and mucus association

In combination, *A. areolatum* and the mucus provide a suitable environment for *S. noctilio* larvae to develop within the tree, although this results in tree mortality (Madden, 1988). Neither the mucus nor *A. areolatum* alone are capable of killing pine trees, but the coalescence of dry areas caused by *A. areolatum* together with the effect of the mucus in the crown, result in the rapid desiccation and death of *S. noctilio* infested trees (Coutts, 1969a). This combined effect produces chlorotic needles, with subsequent needle shedding within 2 to 8 weeks after attack. During drought conditions, the quantity of mucus transferred to the needles could be reduced due to the lack of water being translocated within the tree, while the dormant needles contribute to a reduced water translocation rate. The combination of these effects prevents the movement of mucus up the tree to the needles, where the mucus is effective in the desiccation process. Therefore, the tree would appear more resistant (Madden, 1988).

2.3.5. *Sirex noctilio* interaction with tree resin

When oviposition takes place by the female *S. noctilio*, the tree's defence is to exude resin to close the oviposition hole (Rawlings, 1948). The smell of resin oozing from a tree appears to attract other *S. noctilio* females. The status of resin within the tree will determine the tree's resistance to attack (Rawlings, 1948).

Sirex noctilio epidemics have been associated with periods of drought (Rawlings and Wilson, 1949) since wet wood retards the development of *A. areolatum*. Drought also reduces the resin pressure, which drops to zero when the trees are felled (Coutts, 1965).

Coutts (1965) found resin in the oviposition tunnels, which may be liquid but usually thickens with time and fills the oviposition tunnels. Higher mortality of eggs and first-instar

larvae was recorded in tunnels containing high levels of resin than when resin levels were low. Living trees produce more resin, making them undesirable for successful oviposition by *S. noctilio* (Coutts, 1965).

2.4. Control methods

2.4.1. Tree resistance to *Sirex noctilio*

Research has shown that stressed pine trees are attacked by *S. noctilio* due to the emission of α - and β -terpenes (Simpson, 1976). The emissions of these compounds are because of stress induced by high planting density or competition for nutrients and water. As *S. noctilio* attacks smaller, more stressed trees during subsequent years, these die and are removed from competition (similar to a thinning operation), alleviating the stress on the remaining trees (Madden, 1975).

Natural defence mechanisms of a *S. noctilio*-infested tree include non-preference, antibiosis or tolerance. Non-preference occurs when female wasps reject potential host trees for egg laying. Such trees may or may not be visited by other females who normally display a similar response (Madden, 1977). Antibiosis involves resinosis and polyphenol formation. Resinosis occurs when the wounded tree has a sufficiently high water status and cell turgor pressure for complete infiltration of the cells by resin. The drill holes resulting from *S. noctilio* activity and the surrounding timber are then flooded by resin. Polyphenol formation involves the conversion of photosynthates into fungistatic polyphenols and occurs in response to invasive fungal agents like *A. areolatum*. Tolerance involves the suppression of the mucus effect, either by poor translocation of the mucus to the needles due to drought, or premature senescence and shedding of the affected needles, thereby eliminating the mucus from the tree (Fong and Crowden, 1973).

Silvicultural practices such as spacing, thinning and removal of suppressed trees reduce the number of susceptible trees in a stand. Environmental factors such as drought stress, defoliation and root damage by forestry pests, tree resistance, lightning and over-mature forests trigger a response from the tree and lure *S. noctilio* females to oviposit (Spradbery and Kirk, 1978). Mechanical tree injury during thinning operations, overstocking and poor silvicultural practices that lead to root exposure, flooding, or wounding of standing trees also

ensure the emission of attractants and encourage *S. noctilio* outbreaks (Spradbery and Kirk, 1978). These attractants include monoterpenes and other volatiles that are emitted by the trees (Madden, 1968b). Ovipositing *S. noctilio* females can be lured into artificial traps using a mixture of α - and β -pinene (Simpson, 1976; Spradbery and Kirk, 1978).

Unfortunately, breeding for host resistance against *S. noctilio* attack has, to date, not been successful (Madden, 1988). Similarly, breeding for resistance to the *S. noctilio* mucus has not been achieved. Trees that emit volatiles that attract *S. noctilio* females, but also parasitoids like *I. leucospoides* and *Rhyssa* species may be selected for in future breeding programmes (Bordeaux and Dean, 2012).

In 2007, an international *S. noctilio* symposium and workshop was held in Pretoria and Pietermaritzburg, respectively, in South Africa, during which the international movement of *S. noctilio* and the effectiveness of several biological control measures was considered. This symposium suggested three aspects that needed to be considered and implemented as a complete strategy, namely:

1. Silviculture, to reduce tree stress.
2. The introduction of the nematode *D. siricidicola* in a large inoculation programme, to reduce *S. noctilio* populations.
3. The deployment of other parasitoids such as *I. leucospoides*.

2.4.2. *Deladenus siricidicola*

The parasitic nematode *Deladenus siricidicola* (= *Beddingia siricidicola*) was initially discovered in New Zealand by Zondag in 1962 (Bedding, 1968) in *P. patula* logs that were infested with *S. noctilio*. These nematodes were accidentally introduced into New Zealand through the movement of *S. noctilio* and were present in infested plantations. Parasitism levels by *D. siricidicola* ranging from 75% to 90% were recorded in New Zealand (Zondag, 1969; 1979). In the native range of *S. noctilio* in Europe, hundreds of isolates of seven species of *Deladenus* were collected and screened for selectivity and high rates of parasitism (Bedding and Akhurst, 1978; Bedding and Iede, 2005). These studies resulted in the selection of a *D. siricidicola* strain from Sopron, Hungary that displayed parasitism levels of almost 100% (Bedding and Akhurst, 1974).

A loss of virulence in *D. siricidicola* was first detected in Australia during the Green Triangle *Sirex* outbreak in 1987–1990 (Bedding, 1972; Haugen and Underdown, 1993). This may have resulted from rearing the nematodes in laboratory cultures for over 20 years without them entering the parasitic stage of their life cycle. New cultures of *D. siricidicola* were collected from the Kamona forest in Tasmania, Australia in 1991 for improved virulence in laboratory cultures. This Kamona strain of *D. siricidicola* was subsequently reared and released to replace the initial Sopron strain found throughout Australia (Hurley *et al.*, 2007). The Kamona strain was imported from Australia and released during the mass inoculation programme of 2007, in the KwaZulu-Natal province of South Africa.

2.4.2.1. Nematode life cycle

The nematode parasitizes several siricid wasps, including *S. noctilio*, *S. juvencus* L., *S. cyaneus* F., *S. nitobei* Matsumura and *Xeris spectrum* L.

The life history of *D. siricidicola* involves a free-living or mycetophagous cycle and a parasitic or infective cycle (Bedding, 1967a). The mature fungus-feeding males and females mate for only 10 to 30 seconds, when cultured on potato dextrose agar (Bedding, 1972). Copulation and oviposition takes place between 3°C and 30°C. Egg laying seldom occurs before copulation and unfertilised eggs never develop (Bedding, 1972). The nematodes feed on young *A. areolatum* before and after copulation, and mate more than once (Bedding, 1972). Oviposition occurs within a few hours of copulation with adult females laying up to 1000 eggs and adult nematodes surviving on young *A. areolatum* for several weeks (Bedding, 1972). Eggs hatch after four to five days (at 22°C) and juvenile nematodes consume *A. areolatum* immediately (Bedding, 1972). In the wood, the nematodes take about seven days to mature into mycetophagous adults at 22°C. Older females do not oviposit and retain up to 20 eggs within their oviducts, which hatch and escape through the ruptured cuticle after the female dies (Bedding, 1972).

In *S. noctilio*-infected timber, the juvenile *D. siricidicola* nematodes are restricted in their movement and development by wood moisture content. Once the timber dries to a moisture content below 50% the nematodes start to follow the growth of *A. areolatum*. Juvenile *D. siricidicola* develop into adults and lay eggs in the tracheids within two centimetres of the adult *S. noctilio* oviposition tunnel. Nematodes breed wherever there is sufficient *A. areolatum* and are located in tracheids, between the wood and bark, within host galleries

and in resin canals. All female *D. siricidicola* that feed off young *A. areolatum* lay eggs, which develop into free-living mycetophagous oviparous females or males containing large amoeboid spermatozoa. However, old cultures of *D. siricidicola* may produce infective (parasitic) males and females (Bedding, 1972).

The transition from the free-living mycetophagous form to the infective or parasitic form commences when the nematodes are surrounded by high concentrations of CO₂ and low pH caused by the proximity of *S. noctilio* larvae (Bedding, 1993). In addition, several species of bacteria that have been isolated from *S. noctilio* frass contribute to the development of the infective form (Bedding, 1972). Mating takes place between infective males and females (Bedding, 1967b) or between mycetophagous males and females (Bedding and Iede, 2005). Only fertilised, adult, infective females can enter the *S. noctilio* larvae. The average number of *D. siricidicola* entering a *S. noctilio* larva varies between 5 and 20, but can be as high as 100 (Bedding, 1972).

After penetration into the *S. noctilio* larva, the nematodes migrate into the larva's blood cavity for a few days and undergo a partial moult in addition to rapid growth (Bedding, 1972; Bedding and Iede, 2005). This moult leaves the entire body surface covered in microvilli which absorb food (Riding, 1970). The growth of the nematodes, which can be up to 1 000 times their original volume is, however, dependent on host size and the number of *D. siricidicola* present (Bedding and Iede, 2005). The nematodes reach full size (3 – 25 mm in length) within a few weeks, but the reproductive system of *D. siricidicola* is unaffected until the *S. noctilio* larvae pupate (Bedding, 1972). When infected *S. noctilio* larvae pupate, the *D. siricidicola* ovaries grow rapidly, so that after 5 days the ovaries are half the nematode's length (Bedding, 1972). The ovaries increase to three quarters the nematode's length and produce thousands of eggs (Bedding and Iede, 2005).

After 3 – 4 days, the eggs hatch into juvenile nematodes. The *D. siricidicola* migrate through the tracheids and make use of degenerated bordered pits. Each *S. noctilio* egg contains up to 200 juvenile nematodes, 0.05 mm long, which spread to all parts of the tree where the fungus *A. areolatum* is present as a food source. This mechanism brings the nematodes into contact with the *S. noctilio* larvae that devour the same food (Bedding and Iede, 2005). As *S. noctilio* pupation commences, the adult *D. siricidicola* become packed with juvenile nematodes until their oviducts burst. Juvenile nematodes exit the parent nematode, entering the haemocoel of *S. noctilio* where they migrate to the reproductive organs (Bedding, 1972). Juvenile nematodes enter the ovaries of the female *S. noctilio* prior to the completion of pupation,

causing suppression of ovarian development and reducing egg production. All *S. noctilio* eggs contain juvenile nematodes, which can vary between 50 and 200 (Bedding, 1972). Thousands of juvenile nematodes are thus present in the ovaries and oviducts of infected *S. noctilio*. Any eggs not containing juvenile *D. siricidicola* are usually small and aborted (Bedding, 1972).

Nematode virulence is the measure of effectiveness that is derived by counting the number of adult *S. noctilio* that are infested with *D. siricidicola* (and thereby sterilised) in a given population. Four scenarios exist for poor virulence:

- The nematodes may infect the female *S. noctilio*, but do not infest the eggs (Ryan *et al.*, 2013).
- Where the *S. noctilio* larvae have lowered fat reserves, producing smaller wasps with reduced flight distances (Bedding and Iede, 2005).
- Where artificial inoculations of *D. siricidicola* take place and high infection levels cause *S. noctilio* mortality before pupation, leaving the less virulent nematodes to survive (Slippers *et al.*, 2012).
- The continual laboratory rearing of nematodes in the free-living form, without them passing through the infective form, appears to reduce their virility (Bedding, 1972).

The production of healthy nematodes is paramount to ensure their virulence and survival when artificial inoculations are carried out. Temperature control during their preparation, transport and field application is vital and should be less than 5°C (Bedding and Iede, 2005). The Standard Operating Procedure (Croft *et al.*, 2014) contains the guidelines for the implementation of successful artificial inoculation of *D. siricidicola* into the trees.

2.4.3. *Ibalia leucospoides*, a parasitoid wasp of *Sirex noctilio*

Ibalia leucospoides Hochenwarth (Hymenoptera: Ibalidae) is a solitary koinobiont parasitoid that has been deployed as a biological control agent of *S. noctilio* (Fernandez-Arhex and Corley, 2005). This large wasp, which is 16 – 17 mm long (**Figure 2.1.F**), has been used effectively in a range of climatic conditions (Pietrantuono *et al.*, 2011). These include temperate Mediterranean climates (Australia and southern South Africa), colder climates (Southern Argentina, Chile and Tasmania), and warm semi-tropical regions (Brazil and the summer rainfall areas of South Africa) (Lantschner *et al.*, 2013). In Australia and New

Zealand, 40 % parasitism by *I. leucospoides* has been measured. The parasitoid also occurs throughout most pine plantations in the Southern Hemisphere where *S. noctilio* has been detected (Fernandez-Arhex and Corley, 2005). In drier and warmer regions, *I. leucospoides* appears to perform better due to the lower incidence of prolonged diapause that results in a greater adult emergence (Spradbery and Kirk, 1978).



Figure 2.1.F. *Ibalia leucospoides* released on a *Sirex noctilio*-infested tree.

Figure 2.1.G. *Ibalia leucospoides* insectaria designed to catch numerous adults for redistribution.

Ibalia leucospoides uses olfactory cues to locate *S. noctilio* eggs and larvae. The adults oviposit through the same channels made by *S. noctilio* and the eggs are laid inside the eggs or first-instar larvae of *S. noctilio* (Madden, 1968b). The *I. leucospoides* larva is endoparasitic (feeds internally) until the third instar when it becomes an ectoparasite (feeds externally on the skin) (Fernandez-Arhex and Corley, 2005). The larvae pupate and the adults bore their way out of the tree. After mating, during which 75% of their eggs are mature with the remainder maturing during their adult life, the females initiate oviposition and repeat the cycle. The high female fecundity (about 600 eggs) and their early maturation suggests that the parasitoids are not egg-limited and will achieve population increases (Fischbein *et al.*, 2011; Fischbein *et al.*, 2012). This ability to lay numerous eggs synchronises with the highly variable *S. noctilio* population, allowing *I. leucospoides* to parasitize numerous *S. noctilio* eggs and larvae in periods of high population densities (Fischbein and Corley, 2014).

The longevity of *I. leucospoides* under optimal conditions enhances its biological control potential. Food, when supplied in abundance, can increase adult life span by 42% from the normal 24 day period without food (Fischbein *et al.*, 2012). Adult wasps feed on diluted honey in captivity, but this has not been observed in the field (Pietrantuono *et al.*, 2011). In contrast, (Bernstein and Jervis, 2008) reported that the majority of females consume some food during their adult lifetime, which has a positive impact on their longevity, fecundity and flight activity. In this regard, the establishment of *I. leucospoides* is subject to the availability of natural food being available. Feeding assists in sustaining *I. leucospoides* adults in captivity and during transportation to new release sites, as observed in the SASCP *I. leucospoides* distribution programme in South Africa. However, feeding may not be beneficial prior to field releases as it decreases the wasps' dispersal capacity and increases their time spent on host patches (Pietrantuono *et al.*, 2011). In contrast, (Fischbein *et al.*, 2011) found that feeding *I. leucospoides* did not affect their dispersal capacity or reduce egg production. The only adverse impact appears to be reduced abilities for food and host location (Pietrantuono *et al.*, 2011).

The South Africa *Sirex* Control Programme used insectaria (**Figure 2.1.G**) to harvest *I. leucospoides* in the field for redistribution within the country.

2.4.4. Monitoring the spread of *Sirex noctilio*

Monitoring the wasp's population size, the damage inflicted and the areas affected is an essential element of the control programme. This enables the prioritization of high-risk areas over lower risk areas for control operations and thus the optimal use of resources. In South Africa, the absence of natural enemies of *S. noctilio* has resulted in high population growth rates of the pest in extensive areas of plantation forestry (Wingfield *et al.*, 2001). The monitoring of *S. noctilio* at a landscape level was thus essential to ensure that outbreaks were prevented, and that the biological control interventions were sufficient to control the population.

2.4.4.1. *Sirex noctilio* surveillance

Pest populations, such as *S. noctilio*, require both detection and monitoring. In Australia and New Zealand, formal forest health surveillance takes place to enable early detection of non-

native pests and pathogens (Carnegie, 2008; Wotherspoon, 2008). Such monitoring includes driving or walking through plantation areas or monitoring them with aerial surveillance, using fixed-wing aircraft or helicopters.

Ground-based monitoring surveys undertaken in these countries usually involve the placement of random transects in areas where pests were previously not detected. Such ground surveys, however, need to be intensified to increase the chances of detecting damage. Vantage points are typically used to view the plantations and assist in detecting pest and disease damage (Carnegie *et al.*, 2008).

In comparison, forest managers in the United States of America undertake predominantly aerial monitoring, as this is both efficient and economical in detecting and monitoring changes over large forested areas (Johnson and Wittwer, 2008). This form of detection monitoring incorporates a plot component that provides forest mensuration data, and a survey component that collects pest and pathogen data (Johnson and Wittwer, 2008).

The specific objectives of a Forest Health Monitoring Programme, as defined by the US Forest Health Program (Bennett and Tkacz, 2008), are to:

- Determine detrimental changes or improvements in forests over time.
- Provide baseline and trend information that are statistically precise and accurate.
- Report annually on the status of, and changes in, forest health.

The US program consists of four separate, interrelated activities (**Figure 2.2**):

- Detection monitoring.
- Evaluation monitoring.
- Research on monitoring techniques.
- Intensive site monitoring.

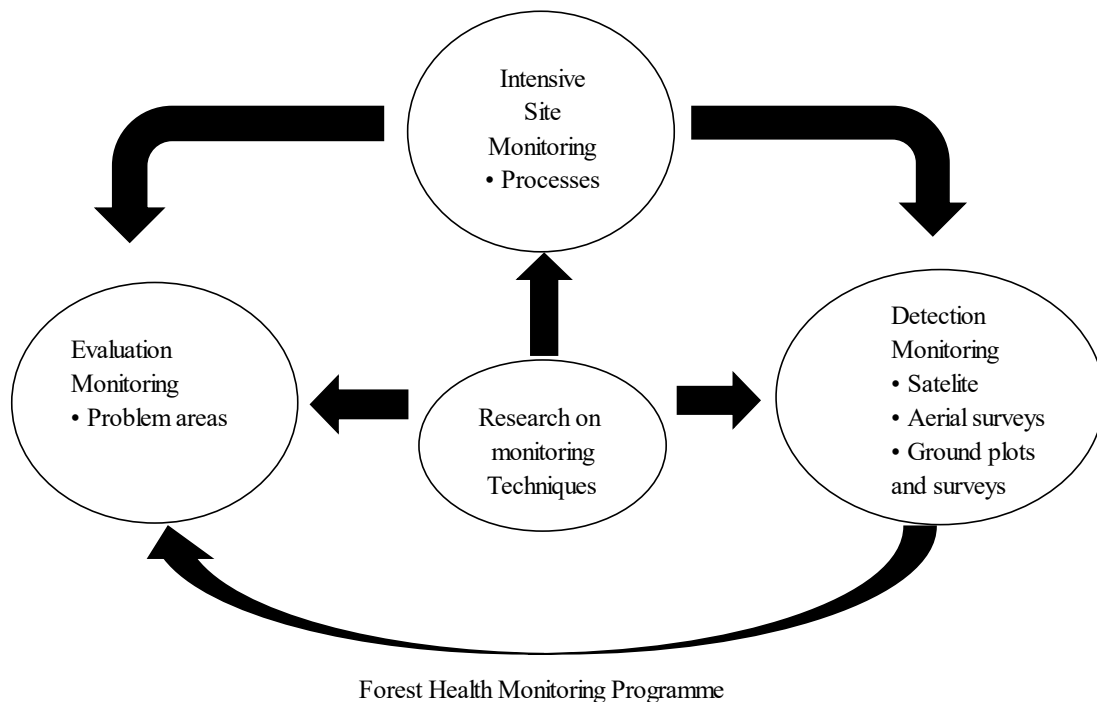


Figure 2.2. Interrelated activities of US Forest Health Monitoring (Bennett and Tkacz, 2008).

Although specifically designed for the US Forest Health system, the integrated monitoring principle is applicable to all plantation monitoring and the same principles were used in the implementation of the SASCP in South Africa. Using this model, the data collected through the SASCP currently provide an annual report on the status of, or changes in, pine plantation health in terms of *S. noctilio* presence and damage. The integrated monitoring programme, as used in the USA, had commonalities with the SASCP that was implemented in South Africa. The SASCP Evaluation Monitoring incorporates three measures derived from the emergence cages, namely verifying parasitism by *D. siricidicola*, determining the number of wasps per log, and measuring the emergence of *I. leucospoides* (**Chapter 3**). The National Monitoring Initiative (NMI) and trap network was the SASCP application of the Intensive Site Monitoring component (as described in **Chapters 4 and 5**). The Detection Monitoring in the context of SASCP incorporated databases in the GIS decision-making tool, where the national land coverage data were used to determine plantation locations, and the NMI and trap network together with the plantation databases and climatic risk model were used to randomise the NMI assessment points (as discussed in **Chapters 4, 5 and 6**). In the SASCP context, satellite and aerial surveys were deemed unable to detect *S. noctilio*-infested trees

and ground surveys were the preferred method (see **Chapter 5**). These four interrelated aspects (**Figure 2.3**) supplied vital knowledge in understanding the current situation and the previous year's impact of *S. noctilio* on forestry in South Africa.

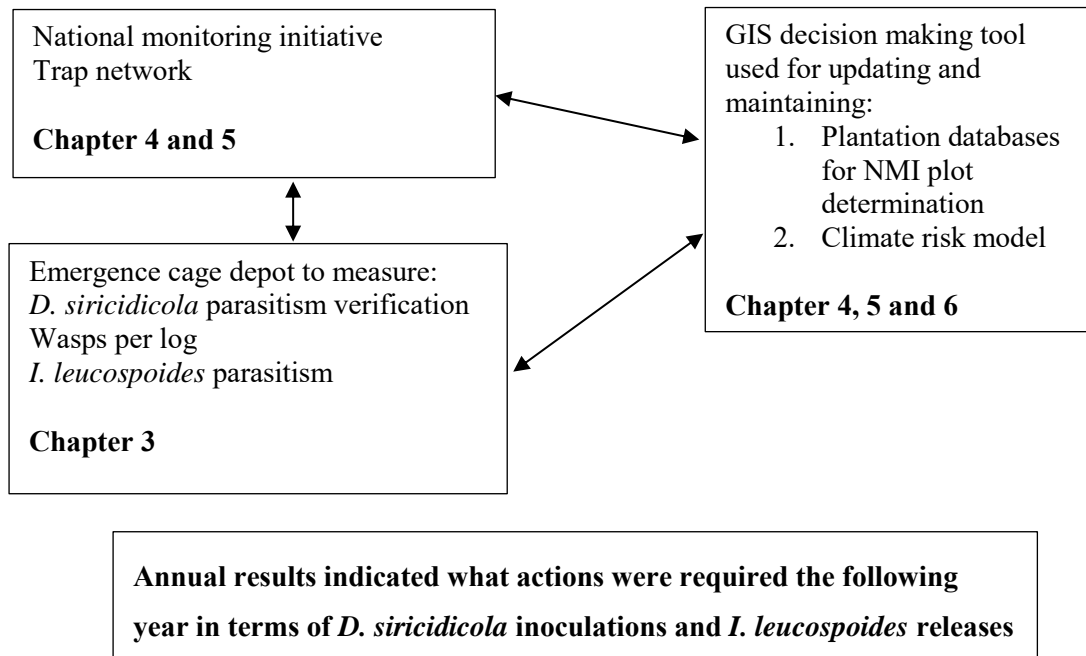


Figure 2.3. The SASCP monitoring structure combining all the data collection elements to create a meaningful result.

In Brazil, trap trees were established by stressing pine trees with herbicide applications and then inoculating them with *D. siricidicola*, which permitted the release of the nematodes without first locating *S. noctilio*-infested trees. These trees were also used for early detection of the pest (Iede *et al.*, 2012). In Australia, a static trapping systems approach was used for detecting *S. noctilio* at low population levels (Bashford, 2008).

Satellite imagery was proposed to monitor forest health in Brazil and identify trees that had been attacked by *S. noctilio*. To date, satellite imagery has made it possible to identify pine plantations affected by various pests and pathogens but it has not been possible to conclusively identify individual trees showing symptoms of *S. noctilio* attack (Iede *et al.*, 2012).

In South Africa, panel traps were initially used to monitor the spread of *S. noctilio* through the country. Once the wasp's presence had been determined throughout the country, the

monitoring method was changed to the use of semi-permanent plots of trees. In these plots, tree mortality was measured and where *S. noctilio*-infested trees were found, these were sampled to determine the parasitism levels at the emergence depots (**Chapter 4 and 5**).

3.0. ANNUAL MONITORING OF PARASITISM OF *SIREX NOCTILIO*

Two introduced parasitic species, which have become established on *S. noctilio* in South Africa, are *D. siricidicola* and *I. leucospoides*. This chapter discusses their effectiveness based on the results of emergence-cage monitoring and each species is considered individually.

3.1. *DELADENUS SIRICIDICOLA*

3.1.1. Introduction

The parasitic nematode *D. siricidicola* has been widely used, both in South Africa and internationally, with large numbers reared in suitable facilities and released in the field.

3.1.1.1. Background to emergence cages

Inoculations with the Kamona strain of *D. siricidicola*, a strain known to be successful in controlling *S. noctilio* in Australia (Bedding and Akhurst, 1978) were initially undertaken in 2004 and 2005 in newly-invaded *S. noctilio* timber. However, following initial inoculations in KwaZulu-Natal province, unexpected low levels of parasitism were obtained (Hurley *et al.*, 2008). This caused the Forestry and Agricultural Biotechnology Institute of the University of Pretoria (FABI), in addition to commercial timber-producing companies, to search for possible reasons for the poor parasitism and control levels achieved. The Industry *Sirex* Trial was thus implemented in 2006 to seek reasons for poor parasitism under South African summer rainfall conditions (Hurley *et al.*, 2008). This involved *D. siricidicola* from various international sources that were inoculated over three time-periods (Hurley *et al.*, 2008) and emergence cages which captured emerging wasps were used to determine the parasitism rates. While this study was in progress, *S. noctilio* populations increased further resulting in increased levels of tree mortality.

Emergence cages are an effective monitoring tool to determine levels of parasitism and were thus used to ensure that *D. siricidicola* was present and active in all pine-growing areas. The use of emergence cages firstly involves the collection of logs infested with *S. noctilio* from

inoculated and un-inoculated timber, which are loaded into the “cages”. The emerging wasps are collected and recorded daily and samples of emerged *S. noctilio* are dissected to record the percentage of parasitism.

In 2007, the results from the Industry Trial (initiated in 2006) revealed that a mass inoculation of *D. siricidicola* would be beneficial in reducing the *S. noctilio* wasp population. To determine the success of the mass inoculation carried out in 2007, emergence cages were used to collect emerging wasps. The same infrastructure that was set up in 2006 for the Industry Trial, at Mondi Linwood Farm, was used to collect *S. noctilio* wasps to test parasitism levels, from both inoculated and un-inoculated trees. All wasps that emerged were sent to FABI for sampling and dissection.

During 2006, there was no facility in South Africa able to produce sufficient nematodes for the 2007 mass inoculation trial and *D. siricidicola* was imported from Australia. Since 2008, FABI was able to produce sufficient juvenile nematodes, with a high rate of survival, for the annual industry *D. siricidicola* inoculation programme.

The results obtained from these emergence cages were beneficial to the forestry industry as they enabled an understanding of the success achieved within the individual forestry companies and provided motivation for future inoculation expenditure. This method was also applicable to the SASCP to benchmark progress in parasitism levels over subsequent years. Therefore, the emergence cages at Mondi Linwood Farm were utilised again in 2008, and annually until 2014 and beyond. All wasps that emerged in the 2006 and 2007 sampling years were sent to FABI, who devised a sampling strategy to determine the number of wasps to be dissected. From 2008 onwards, wasp sampling was carried out at the Linwood depot, and only the samples of wasps that needed to be dissected were sent to FABI.

In the use of emergence cages, the complex ownership of commercial forestry in South Africa must be considered, since approximately 10 large timber companies and many smaller growers all required parasitism information at a regional and farm level to enable them to manage *S. noctilio* effectively on their land holdings. These parasitism data have been used to assist in future control planning (**Figure 3.1**).

Sampling of plantations for emergence cage logs was implemented based on the companies with the highest risk, these being pulpwood producers, who took full advantage of the facilities. The results are therefore skewed towards pulpwood growers although sawtimber companies have not experienced the same high-risk situation with high tree mortality. The sampling has given the SASCP good information to reduce the threat of *S. noctilio* across South Africa, but the sawtimber companies have not received an equivalent benefit. Another important aspect of the emergence cages relates to the capture of *D. siricidicola* from *S. noctilio* for reintroduction into *D. siricidicola* rearing to prevent a deterioration in virulence within the *D. siricidicola* cultures.

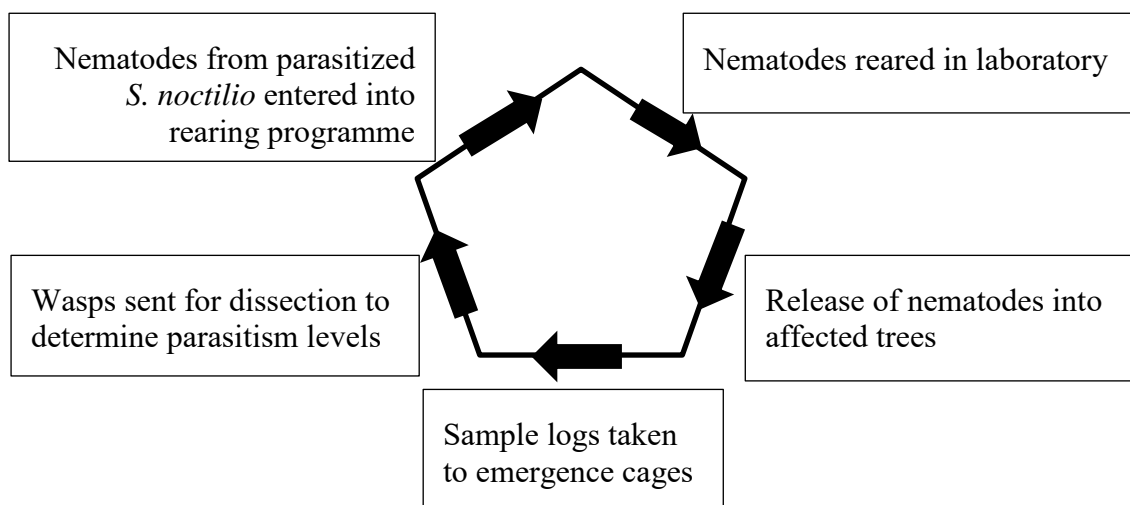


Figure 3.1. *Deladenus siricidicola* production cycle must include the step where a *Sirex noctilio* female is parasitized, to retain parasitic ability.

3.1.1.2. Aim of emergence cages

The system of emergence cages has enabled the collection and sampling of emerged wasps, from logs collected on various plantations, to determine the status of *D. siricidicola* parasitism. Knowledge of parasitism levels at various landscape levels such as regional, plantation, farm or compartment level, aids in directing management decisions for future preventative action.

The initial aim of the emergence cages was to determine the effectiveness of inoculations and to measure background parasitism levels. Over time, the levels of background parasitism and inoculation parasitism become obscured as wasps carrying nematodes lay eggs in trees that have been inoculated with nematodes, thus increasing the levels of parasitism. The

parasitism levels obtained were an indication of what was happening in the plantation and were therefore vital for planning follow-up inoculations. For example, compartments with a low level of parasitism would require additional *D. siricidicola* inoculation if tree mortality remained high, whereas if the parasitism level was high (>10%) (Bedding, 2009), and there was a reduction in tree mortality, no further inoculations were required as the background parasitism would be expected to increase over subsequent years. Thus, increases or decreases in parasitism needed to be continually monitored to ensure the maintenance of parasitism across the plantation or region.

3.1.1.3. South African *Sirex* Control Programme requirement

The South African *Sirex* Control Programme (SASCP) required information for pine afforested areas across the entire country so that it could focus inoculation efforts in areas where parasitism was poor or still absent. Large landowners growing pine trees were represented in the emergence cages programme, but for smaller and non-represented land owners the SASCP used the results from neighbouring farms or companies to advise these non-represented land owners on predicted parasitism rates. In this manner, the SASCP assisted small growers in developing an appropriate inoculation programme to address the threat of *S. noctilio* on their land holdings.

3.1.1.4. Background to wasp sampling

A wasp sampling strategy was developed to reduce the number of wasps that needed to be dissected for confirmation of parasitism. Wasp dissection remains a time consuming and costly process since it involves pinning the wasp to a board, dissecting the egg chamber of the female, removing the eggs onto a microscope slide, and examining the broken eggs for *D. siricidicola*. In males, the testes are dissected and checked for the presence or absence of *D. siricidicola*. The presence or absence of *D. siricidicola* in each sample is then recorded. If *D. siricidicola* is present, then they are collected, labelled and stored for future breeding.

In 2006, the Industry Trial required that all wasps collected from the cages had to be dissected. In 2007 and 2008, all females and 5% of the males that were collected every second day, were sent weekly to FABI for dissection. The males that were collected on other days were counted and destroyed. In 2009, the sampling strategy was changed so that all

females and a maximum of five males collected per cage per week were sent to FABI for dissection. In 2010, the sampling was changed again with five males per cage per week, for the first 100 emerging male wasps, sent to FABI. If between 100 and 200 males emerged, then the sample size was increased to 10 males per cage per week. The females were also sampled and a maximum of 10 females per cage per week were sent to FABI for dissection. This prevented the loss of data relating to males which emerged from cages, only on days when all the wasps had been killed according to the previous sampling strategy. In 2011, the sampling strategy was further refined from five males down to three males per cage per week. The female sampling remained at 10 females per cage per week. This sampling strategy was maintained in 2012, 2013 and 2014.

Sampling female wasps was ideal in that *D. siricidicola* were collected from the dissected *S. noctilio* eggs, which indicated successful parasitism. These nematodes were collected, reared and then released into affected trees through inoculation. Male wasps, if parasitized, have *D. siricidicola* present in the testes that could be collected and stored for future use, but *D. siricidicola* are not transferred to the females during mating. These *D. siricidicola* in males do not assist in reducing the wasp population. However, when the logs are placed in emergence cages either males or females or both sexes emerge from the logs. Therefore, it was necessary to dissect a percentage of male wasps because there were occasions when no, or only a few, female wasps emerged and a result based on relatively few wasps would not be robust.

3.1.1.5. Number of wasps per log

The number of wasps per log reflected two situations that occur during the flight season, when the female *S. noctilio* wasps were seeking suitable host trees for oviposition. The first situation occurred during the 2010 drought period in the George area in the Western and Eastern Cape Provinces, where there were many water-stressed trees. During this time, the *S. noctilio* females selected many different trees into which they oviposited, resulting in few wasps emerging per log. In other years when average and above-average rainfall was recorded, the females sought stressed trees, which were fewer in number and more difficult to find, and under such conditions, more eggs were laid in fewer trees. The second situation was when the *S. noctilio* population was decreasing due to adequate control by *D. siricidicola* and *I. leucospoides*, with fewer eggs deposited per tree or per log due to the smaller

population size. Therefore, with an understanding of the climatic conditions and parasitism levels within a region, a reduction in the number of wasps per log was considered to be a reasonable indication of a reduction in the *S. noctilio* population.

Another factor that was independent of the *S. noctilio* population but that impacted on the number of *S. noctilio* wasps that emerge per log was increasing populations of *I. leucospoides*. The larvae of *I. leucospoides* kill *S. noctilio* larvae and eventually emerge as adults. The emergence cages also collected *I. leucospoides* that emerged with *S. noctilio* and together indicated the entire population of *S. noctilio* that was present in the logs.

3.1.1.6. Size of depots

The size of the depot was determined by the number of cages that were required to test the background and inoculated parasitism levels for the region served by the depot. The restraint on the number of cages was the number of wasps that emerged, and although reduced through a sampling strategy, the wasps required dissection to obtain their parasitism status. The nematodes collected from dissected wasps are stored per their cage number. Once all dissections were completed, nematodes that produced high levels of parasitism were cultured for further field inoculation. Over the past few years, FABI have dissected over 10 000 wasps per year, during the short flight season. The number of cages deployed (**Table 3.1**) was the minimum number required to service the needs of the 10 corporate companies and private growers, while remaining within the wasp dissection parameter.

Table 3.1. Number of utilised cages at each *Sirex* emergence depot

Year	2007	2008	2009	2010	2011	2012	2013	2014
Linwood	600	573	387	243	153	156	156	174
George				123	108	108	97	108
Vryheid				123	276	393	444	453
Total	600	573	387	489	537	657	697	735

The risk ratings (**Chapter 6**) indicated a wide range of risk categories across most plantations and farms. Therefore, on the fragmented plantations and farms, each area of high risk needed to be monitored to determine the parasitism status. If the farms and plantations were more

homogenous in risk, then fewer samples were taken which reduced the number of cages required.

3.1.2. Materials and Methods

3.1.2.1. Quality control of *D. siricidicola*

For successful inoculation, it was important that the *D. siricidicola* purchased were healthy juvenile nematodes as this had a direct effect on the levels of parasitism. In 2007, *D. siricidicola* was imported from Australia, but due to time in flight, customs handling, and further transport to plantation, survival was reduced to 50%. Since 2008, FABI has supplied the Kamona strain of *D. siricidicola* to reduce the supply-chain time.

Each sachet, now changed to a plastic bottle for practical reasons, contains five million juvenile *D. siricidicola* in approximately 45 ml of water. At the delivery point, a 10% sample of the number of bottles in the consignment is checked (Croft *et al.*, 2009). The procedure for counting nematodes appears in the Standard Operating Procedure (Croft *et al.*, 2014). *Deladenus siricidicola* survival was above 95% and often reached 99% at the delivery site. The number of living juvenile nematodes per sachet should be five million. The actual supply of *D. siricidicola* received from FABI was often between five and seven million living juvenile nematodes per bottle, giving on average a 132% supply on order (**Table 3.2**).

Table 3.2. *Deladenus siricidicola* survival and supply on order measured at the delivery destination

Year	No. of bottles	No. of nematodes ordered	No. of nematodes supplied	Actual survival %	Survival on order %
2008	623	3 115 000 000	3 845 779 000	96	123
2009	166	830 000 000	1 150 048 000	98	139
2010	100	500 000 000	518 402 850	88	104
2011	216	1 080 000 000	1 639 373 538	95	152
2012	200	1 000 000 000	1 439 388 235	95	144
2013	198	990 000 000	1 279 514 323	99	129
2014	173	865 000 000	1 046 623 385	99	121
2015	195	975 000 000	1 147 987 941	99	118
Total	1871	9 355 000 000	12 067 117 272	96	129

3.1.2.2. Inoculation methods

In South Africa, the Australian method (Bedding and Akhurst, 1974) of felling trees for the inoculation process on sawtimber trees was used, and a standing tree technique was developed to inoculate pulpwood trees between 10 and 18 years of age (Verleur, 2009). A standard procedure was developed whereby any tree over 18 years of age was felled and then inoculated, while those trees younger than 18 years were inoculated while standing. This was applied as standard practice unless otherwise specified by the landowner due to his safety guidelines or preference.

3.1.2.2.1. Trees inoculated while standing

The standing tree inoculation technique (**Figure 3.2**) involved the placement of four inoculation holes at knee height (± 50 cm) from the ground and another four holes at 30 cm above the first hole in four lines up the tree. This technique was repeated eight times while standing on the ground. To reach further up the tree, a 3 m ladder was used and two holes were placed on either side of the tree at 30 cm intervals going up the tree nine times (30 cm above the point where the four holes end). This procedure thus provides 50 inoculation holes per tree. A small amount of the inoculum, approximately 10 ml containing *D. siricidicola*

and hydrogel, was placed into each of the holes allowing the nematodes to move into the tracheid and locate the *S. noctilio* larvae.

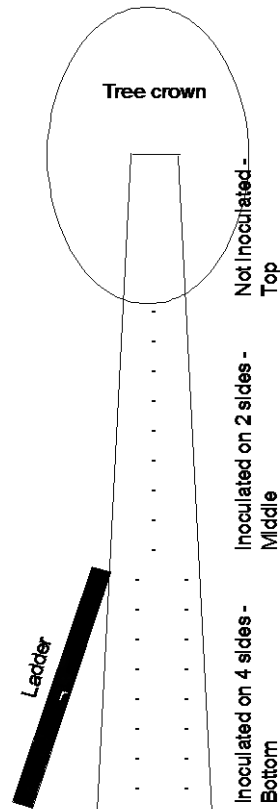


Figure 3.2. Illustration of the inoculation method on standing trees and the areas from which background logs were collected to measure parasitism

3.1.2.2.2. Trees inoculated after felling

The inoculation of large trees, however, required trees to be felled and inspected for resin droplets indicating where the female *S. noctilio* had laid her eggs. Where these resin droplets started, the inoculation was initiated and continued up the tree until the resin droplets ended. This ensured that the portion of the tree that had been utilised by the wasp was inoculated with *D. siricidicola*. Two lines of inoculation holes (**Figure 3.3**) were made up the tree at 30 cm intervals until the diameter of the tree was less than 10 cm, and then one hole was made at 30 cm intervals. The trees were inoculated as before (see above).

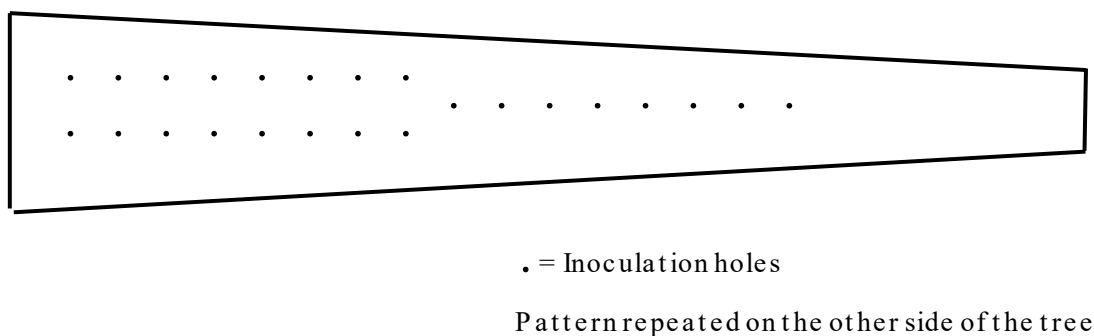


Figure 3.3. Placement of inoculation holes on a tree felled for inoculation with *Deladenus siricidicola*.

3.1.2.3. Log sampling

3.1.2.3.1. Log collection and sampling

To manage the SASCP programme effectively, an accurate annual estimate of parasitism by regions, by company and by landowner was needed. Therefore, the sample log collections for the emergence cages needed to represent each component of the forestry industry.

Knowledge of the tree species and regimes under which the timber was grown was also necessary since pulpwood regimes had more highly stocked compartments (e.g. 1666 stems per Ha (spHa)) where competition for water was greater, placing smaller trees under water stress. Sawtimber plantations were, however, thinned (e.g. 400 spHa), which reduced competition for water, and thereby water stress. *Sirex noctilio* prefers water-stressed trees, which made pulpwood plantations more vulnerable, due to the high tree stocking.

Parasitism for this component of the study was expressed as the percentage of emerged female and male wasps that were infected with *D. siricidicola*. To obtain the *S. noctilio* wasps, logs infested with *S. noctilio* larvae were collected and placed into emergence cages. The ‘emergence cages’ used by the SASCP consisted of 210 L drums with the open end closed with a removable net (**Figure 3.4**). The nets allow the logs to be loaded into the cages and the collection of the emerged wasps.

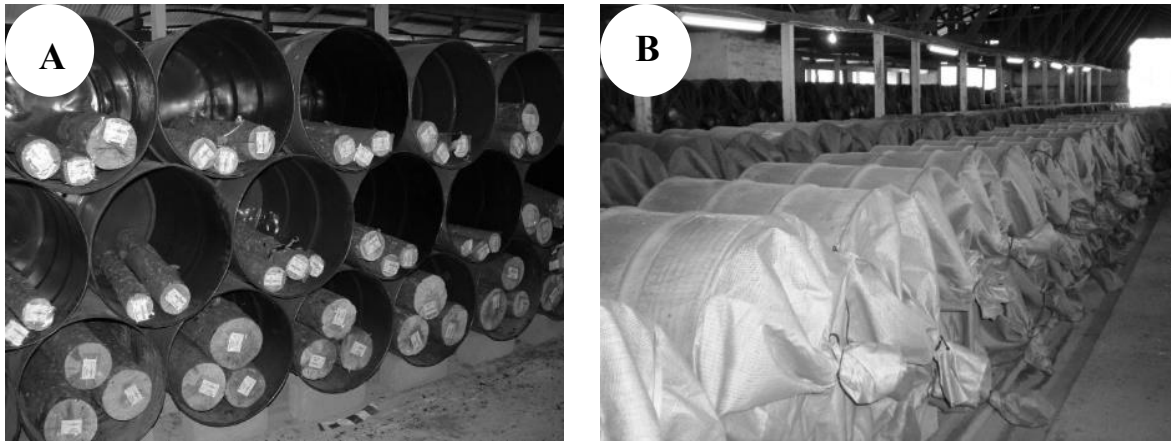


Figure 3.4. Emergence cages loaded with logs (A) without, and (B) with nets.

The SASCP needed to provide an estimation of parasitism for the entire country, yet remain within an optimal number of logs and cages. To accommodate this, a sampling method was developed to provide a representative estimate of *D. siricidicola* parasitism at various plantation-sampling intensities that would enable the industry to manage *S. noctilio* populations.

The number of logs collected should accurately represent the mature plantation area associated with the sample. Logs were collected from plantation areas, which ranged from low to high populations of *S. noctilio*, in addition to low to high risk areas based on climatic factors (**Chapter 6**). The results reflected the number of wasps that emerged per log and the associated parasitism levels. In some areas, high levels of parasitism were obtained together with high numbers of wasps that emerged from the logs, although only a few trees from the sampled area were attacked by *S. noctilio*. In contrast, there were areas where more trees were affected by *S. noctilio* but the numbers of wasps per log were low, with good levels of parasitism measured. This may have been influenced by climate and the resultant climatic and silvicultural-induced stress levels.

3.1.2.3.2. Log sampling from inoculated standing trees

Logs were collected from compartments where *S. noctilio*-infested standing trees had been inoculated. Three inoculated trees that were representative of the compartment were selected and felled. Each log section was inspected to ensure that *S. noctilio* larvae were present. If larvae were absent, then another log section was selected. An 80 cm section was removed from the bottom section where the tree had been inoculated on four sides. The middle log

was removed from the tree where it had been inoculated on two sides. The third log was removed from the next section up the tree where no inoculations had taken place (see **Figure 3.2**). The three bottom logs were placed in one emergence cage; the three middle logs were placed in a second emergence cage and the three top logs placed in a third emergence cage. These collections comprised one sample. If many trees had been inoculated in a compartment, then this process was repeated two or three times (i.e. using six or nine trees). (**Figure 3.5**).

3.1.2.3.3. Log sampling from un-inoculated standing trees infested with *S. noctilio*

Un-inoculated trees were used to determine the background or natural parasitism where *S. noctilio* had transported *D. siricidicola* from one tree to another tree and the emerging wasps were parasitized. The tree sampling process for inoculated felled trees (section 3.1.2.3) was followed for un-inoculated trees, and sample logs were placed in emergence cages to determine background parasitism levels. A similar number of trees was selected for each site, as for the inoculated trees. It was important that the log sample was infested with *S. noctilio*.

The preparation of the sample logs included inspections of the tree for signs of *S. noctilio* infestation such as a dead tree crown, dry timber, presence of females or ovipositor evidence, resin droplets indicating oviposition, and *S. noctilio* larvae in recently made tunnels. To achieve this, each sample tree was cross-cut at the lowest point within the tree section and discs were cut until there was clear evidence of *S. noctilio* larval tunnels. From that point, an 80 cm section was measured and removed from the tree. Where there was no evidence of *S. noctilio* larval tunnels, then another tree was selected.

3.1.2.3.4. Log sampling from inoculated felled trees

Log samples were collected from pine compartments where the trees had been felled and then inoculated. Three inoculated trees were selected with an 80 cm section removed from the bottom, middle and top thirds of the tree (see **Figure 3.5**). Each log section was inspected for the presence of *S. noctilio*. If there were no signs of *S. noctilio*, then another sample was taken until a log containing *S. noctilio* larvae was found. The three bottom, middle and top logs were placed in separate emergence cages as before to comprise one sample. Where

many trees had been inoculated in a compartment, this process was repeated two or three times (using six or nine trees).

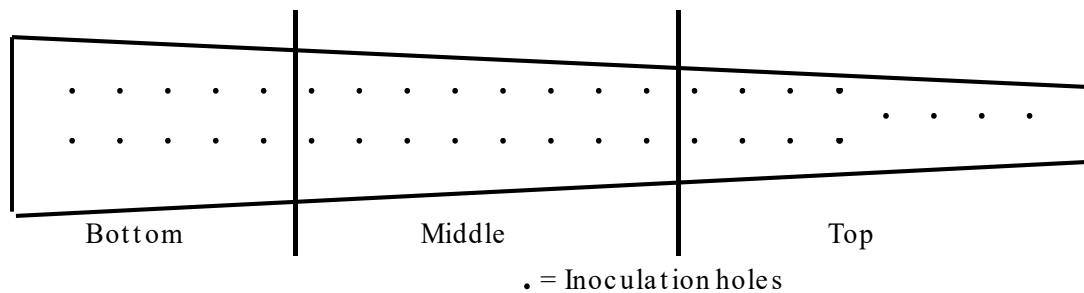


Figure 3.5. Areas from which logs were sampled from inoculated felled trees for parasitism verification

3.1.2.4. Collection and sampling of emerging *S. noctilio* wasps

The emergence cages were monitored daily for emerging wasps. Once the first wasp of the emergence season had emerged, a daily routine was carried out whereby male and female wasps were collected in separate bottles, from each emergence cage. Once all the cages had been checked and the wasps removed, the numbers of wasps per cage were recorded. The emerged wasps were stored at between 5 and 10°C. At the end of each week, a sample of wasps from each cage were sent to FAB I for dissection and assessment of parasitism. This process was continued until the end of the emergence period.

3.1.2.5. Three tier sampling strategy

Three sampling strategies were necessary; initially, plantation tree selection, then log sampling and finally, wasp sampling.

The first level of sampling occurred at the plantation level where trees were selected from the plantation, or block or compartment. Plantations varied in relation to size, age class, pine species and climatic risk. Only *S. noctilio* infested trees were selected and included inoculated trees and un-inoculated trees to determine inoculated and background parasitism levels, respectively. The number of trees sampled was dependent on the number of trees that were inoculated. If many trees were inoculated, then up to nine trees were sampled to provide three logs each from the bottom, middle and top sections of the trees. Additional samples were sometimes taken from another section of the farm which was inoculated. From

the thousands of trees on a farm, we reduced the sample to a maximum of nine trees, and in extreme cases, 18 trees. The second level of sampling involved the logs within each tree. In pulpwood trees, which ranged from 13 to 18 m at an age of 15 to 18 years, three 80 cm long logs were taken from three different height sections (see above). This represents 13% to 18% of the sampled trees. The third level of sampling occurred when the wasps emerged from the logs. The male and female wasps were collected daily, counted and recorded. The wasps were sampled at weekly intervals and 10 females and three males per cage per week were dissected to record parasitism by *D. siricidicola*. This sampling that included over 735 cages (**Table 3.1**) equated to around 10 000 wasps that were dissected per year. This sampling strategy changed from 2007 to 2014 in terms of the weekly numbers per cage that were sent to FABI for dissection, ending in 10 females and 3 males, per cage, per week.

Reporting on these results also took place at several levels. The depot results showed percentage parasitism and trends over successive years. Within each depot, the results from each region and forestry company were determined. When three cages from a compartment were considered in relation to the number of wasps that emerged and those that were parasitized, the results could not be statistically verified due to the variation in the number of wasps that emerged. Emerging wasps ranged from a few to over 300 per cage on a few occasions, although most cages delivered low numbers of wasps. Frequently, no females emerged from a cage and only males were included in the dissection sample.

During the emergence period, parasitism percentages increase over time as do the numbers of emerging wasps. For this reason, the sampling strategy should not be changed during a flight season / emergence period should the sample size be considered as too small. The emerged wasps could not be stored successfully for longer than a week prior to dissection. Therefore, it was better to dissect more wasps, collected on a weekly basis, to determine with some accuracy the parasitism percentage on a reasonable number of wasps from each cage. To statistically verify the cage level results, more samples and wasps would have needed to be collected which increased the cost and work load of the people dissecting the wasps. Depot, regional and company reporting provided sufficient information for a national programme.

3.1.3. Results and Discussion

3.1.3.1. Numbers of dissected wasps

Wasps were recovered from all three depots, with samples sent to FBI for dissection and the remainder destroyed. From 2007 to 2014, the 735 emergence cages at the three depots recovered huge numbers of wasps that required dissection (**Table 3.3**). The numbers of wasps dissected from all three depots fluctuated annually, depending on the number of wasps that emerged per log.

Table 3.3. Numbers of emerged and dissected *Sirex noctilio* wasps over an 8-year period

	Depot Year	No. of emerged wasps			No. of dissected wasps			Dissected of total (%)		
		Male	Female	Total	Male	Female	Total	Male	Female	Total
		55848	10563	66411	20105	10053	30158	36	95	45
Linwood	2007	19907	3078	22985	5942	2886	8828	30	94	38
	2008	10237	1991	12228	1979	1929	3908	19	97	32
	2009	9763	1652	11415	4958	1573	6531	51	95	57
	2010	4491	757	5248	2784	752	3536	62	99	67
	2011	2397	880	3277	1160	862	2022	48	98	62
	2012	1929	794	2723	951	751	1702	49	95	63
	2013	3303	599	3902	958	525	1483	29	88	38
	2014	3821	812	4633	1373	775	2148	36	95	46
		19356	5009	24365	13272	4857	18129	69	97	74
George	2010	5914	1608	7522	4474	1592	6066	76	99	81
	2011	5442	1203	6645	3147	1201	4348	58	100	65
	2012	3103	798	3901	1836	683	2519	59	86	65
	2013	2611	810	3421	2275	807	3082	87	100	90
	2014	2286	590	2876	1540	574	2114	67	97	74
		78065	11451	89516	17411	10673	28084	22	93	31
Vryheid	2010	4901	871	5772	2147	849	2996	44	97	52
	2011	15199	2774	17973	3061	2647	5708	20	95	32
	2012	19867	3047	22914	4223	2774	6997	21	91	31
	2013	22831	2634	25465	3969	2387	6356	17	91	25
	2014	15267	2125	17392	4011	2016	6027	26	95	35
Total	153269	27023	180292	50788	25583	76371	33	95	42	

Sirex noctilio has a skewed sex ratio ranging from 5-32 males for every female, with the average female depositing around 250 eggs (Iede *et al.*, 1998). The depot data (**Table 3.3**) was used to calculate the sex ratio (**Table 3.4**) based on the number of emerged wasps. Each

emergence depot has a unique sex ratio range, which ranged from 2.4 to 8.7 males for every female, which was often below that reported in the literature (Iede *et al.*, 1998).

Table 3.4. Emerged *Sirex noctilio* sex ratio based on emerged wasps

	Depot	Total emerged wasps			Sex Ratio
	Year	Male	Female	Total	Males / Female
Linwood		55848	10563	66411	5.3
	2007	19907	3078	22985	6.5
	2008	10237	1991	12228	5.1
	2009	9763	1652	11415	5.9
	2010	4491	757	5248	5.9
	2011	2397	880	3277	2.7
	2012	1929	794	2723	2.4
	2013	3303	599	3902	5.5
	2014	3821	812	4633	4.7
		19356	5009	24365	3.9
George	2010	5914	1608	7522	3.7
	2011	5442	1203	6645	4.5
	2012	3103	798	3901	3.9
	2013	2611	810	3421	3.2
	2014	2286	590	2876	3.9
		78065	11451	89516	6.8
Vryheid	2010	4901	871	5772	5.6
	2011	15199	2774	17973	5.5
	2012	19867	3047	22914	6.5
	2013	22831	2634	25465	8.7
	2014	15267	2125	17392	7.2
Total	153269	27023	180292	5.7	

The dissection of male and female wasps enabled us to collect *D. siricidicola* from the eggs or testes, but population reduction is brought about through the parasitized females.

Therefore, the sampling strategy was to sample a higher percentage of females (**Table 3.3**).

A proportion of males were dissected to provide estimations of parasitism so that when no or few females emerged from a cage, parasitism was calculated on a reasonable sample size.

Table 3.5. Total numbers of emerged and dissected wasps at all three depots over the 8-year period

Years	No. of emerged wasps			No. of dissected wasps			Percentage dissected		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
2007	19907	3078	22985	5942	2886	8828	30	94	38
2008	10237	1991	12228	1979	1929	3908	19	97	32
2009	9763	1652	11415	4958	1573	6531	51	95	57
2010	15306	3236	18542	9405	3193	12598	61	99	68
2011	23038	4857	27895	7368	4710	12078	32	97	43
2012	24899	4639	29538	7010	4208	11218	28	91	38
2013	28745	4043	32788	7202	3719	10921	25	92	33
2014	21374	3527	24901	6924	3365	10289	32	95	41
Total	153269	27023	180292	50788	25583	76371	33	95	42

Between 2007 and 2014, 42% of the total numbers of emerging wasps at all three depots were dissected (**Table 3.5**) which comprised 95% of the emerging females and 33% of the males. Despite this high proportion, parasitism levels in many cages were calculated from a small number of emerging wasps (<5 per cage). These parasitism results determined the following year's inoculation plan, which carried high costs. Incorrectly calculated parasitism based on small numbers could result in unnecessary expenditure on *D. siricidicola* inoculation.

3.1.3.2. Parasitism of *S. noctilio*

The presence of *D. siricidicola* at each of the depots was expressed as a percentage of the total number of wasps that were dissected per cage.

Parasitism results at the Linwood depot level are indicated in **Figure 3.6**. Linwood was the first depot to be created in 2006 and was followed by the Vryheid and George depots in 2010. The Linwood depot served KwaZulu-Natal and the northern part of the Eastern Cape Province, while Vryheid represented northern KwaZulu-Natal, Mpumalanga and Limpopo Provinces, with the George depot covering the Western Cape and southern part of the Eastern Cape Province. Initially, the timber that would have been sent to the Vryheid depot in 2009 was sent to the Linwood depot and this contributed towards the low levels of parasitism at the

depot. This was because initial inoculations yielded lower levels of parasitism, which built up over time. Increased parasitism was due to the inoculations of *D. siricidicola* and the background movement of *D. siricidicola* because of infected *S. noctilio* converging in the same trees.

The intention of the SASCP was that the background parasitism should increase, removing the necessity for continued inoculations of *D. siricidicola* and ensuring a natural system. Inoculations remained artificial; for example, in KwaZulu-Natal, Mpumalanga and Limpopo Provinces the earliest detection of *S. noctilio* presence in the trees was mid-February when the crowns discoloured. Natural inoculation of *D. siricidicola* into the trees was during egg laying which took place in November and December of the previous year.

Therefore, once the Vryheid depot had opened, an increase in the parasitism rate at Linwood was a true reflection of the region that the Linwood depot represented (**Figure 3.6**), namely the northern part of the Eastern Cape and KwaZulu-Natal Provinces. The area represented by the George depot had no emergence cages prior to 2010.

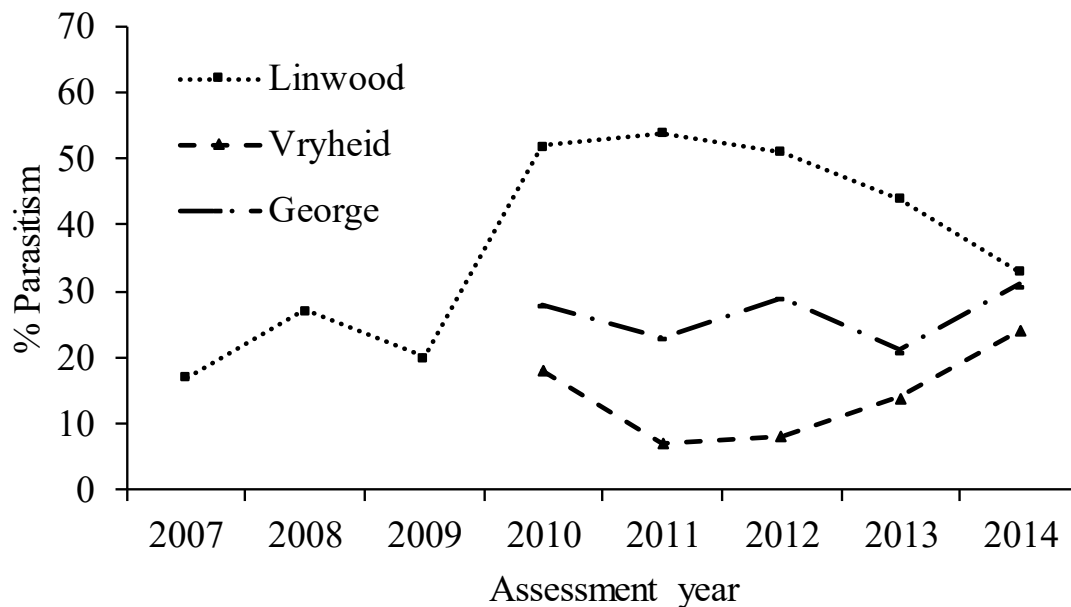


Figure 3.6. Percentage parasitism recorded at the three depots from 2007 to 2014.

Had the parasitism at the Linwood depot been calculated on the logs that represent that region, (excluding those which could have been in the Vryheid depot), then the Linwood total parasitism would have been around 4% higher (**Figure 3.6**).

The Linwood depot was located outside Pietermaritzburg, in KwaZulu-Natal. The large 2007 inoculation program with imported *D. siricidicola* resulted in 16% male and 19% female parasitism, with an overall parasitism level of 17% (**Figure 3.6**). In 2008, further inoculations took place in KwaZulu-Natal and, in many instances, on the same farms as in 2007. This resulted in 20% male and 33% female parasitism, resulting in an overall parasitism level of 27%. This trend of increased parasitism occurred at all depots over subsequent years of inoculations (**Figure 3.6**). The decreasing levels of parasitism at Linwood from 2011 to 2014 was because no further inoculations had been undertaken in KwaZulu-Natal province since 2011.

The range of parasitism levels (**Table 3.6**) shows that annually achieved results ranged from 0% to 100% at the cage level, except at the George depot in 2011 and 2013 where upper limits of 88% and 79%, respectively, were achieved.

Table 3.6. Minimum and maximum parasitism levels achieved (at the cage level) at all three depots.

Depot	Year	No. of cages	% Males parasitized		% Females parasitized	
			Minimum	Maximum	Minimum	Maximum
Linwood	2007	600	0	100	0	100
Linwood	2008	573	0	100	0	100
Linwood	2009	387	0	100	0	100
Linwood	2010	243	0	100	0	100
Linwood	2011	153	0	100	0	100
Linwood	2012	156	0	100	0	100
Linwood	2013	156	0	100	0	100
Linwood	2014	174	0	100	0	100
George	2010	123	0	100	0	100
George	2011	108	0	88	0	100
George	2012	112	0	100	0	100
George	2013	96	0	79	0	100
George	2014	108	0	100	0	100
Vryheid	2010	123	0	100	0	100
Vryheid	2011	276	0	100	0	100
Vryheid	2012	393	0	100	0	100
Vryheid	2013	444	0	100	0	100
Vryheid	2014	453	0	100	0	100

Although, *D. siricidicola* could parasitize up to 100% of the *S. noctilio* wasps when considering parasitism at the emergence cage level, these figures are misleading. A more important indicator was the frequency at which the various levels of parasitism occurred. The frequency of parasitism was calculated using individual cage parasitism percentages that were assigned to a percentage class, with frequency indicated by the number of cages in each class. At the Linwood depot in 2007, the frequency of parasitism at the cage level was predominately in the lower percentage range (**Figure 3.7**). In 2007, wasps that emerged from 242 cages yielded 0% parasitism. Although five cages produced 100% parasitism in both males and females, these cages produced few wasps, (2, 7, 12, 14, and 43 male and female wasps over the emergence season) and due to the sampling strategy, a maximum of 10 wasps from one cage were dissected.

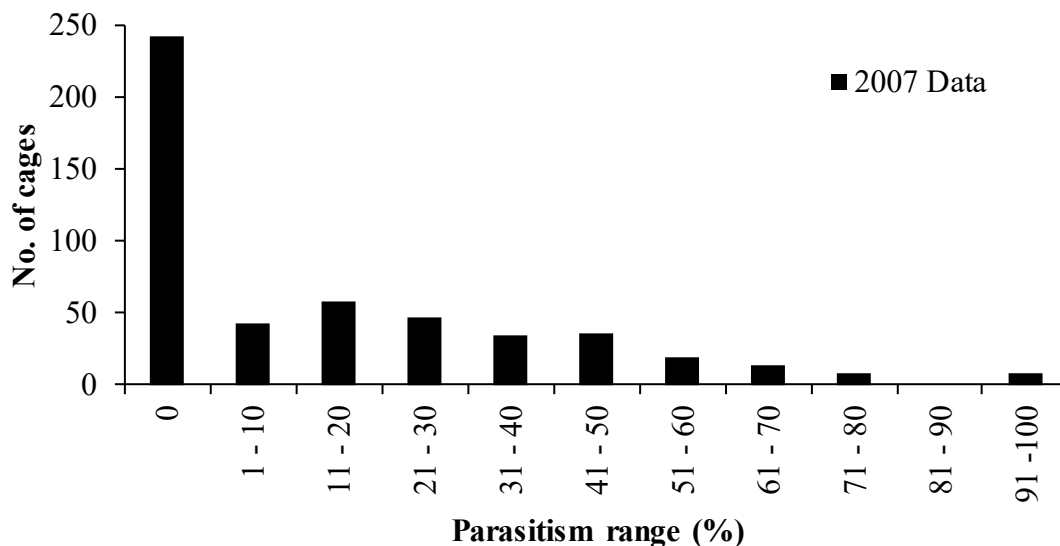


Figure 3.7. Frequency of parasitism at the Linwood depot in 2007, indicating the number of cages within each percentage parasitism class

By 2014, the frequency at which parasitism occurred, as observed at the cage level at the Linwood depot, had improved (**Figure 3.8**). In 2014, no parasitized wasps emerged out of 67 cages, which was considerably better than in 2007 (**Figure 3.7**). The frequency of parasitism increased due to increased inoculations, with slightly higher numbers of cages in the 31% to 40% category. The number of wasps that emerged per log ranged from 0 to 29 males and 0 to 16 females, with average numbers of 7.8 males and 1.7 females per log. In 2007, for comparative purposes, the number of wasps that emerged per log ranged from 0 to 85 males and 0 to 13 females, with the average number of 11.5 males and 1.8 females per log.

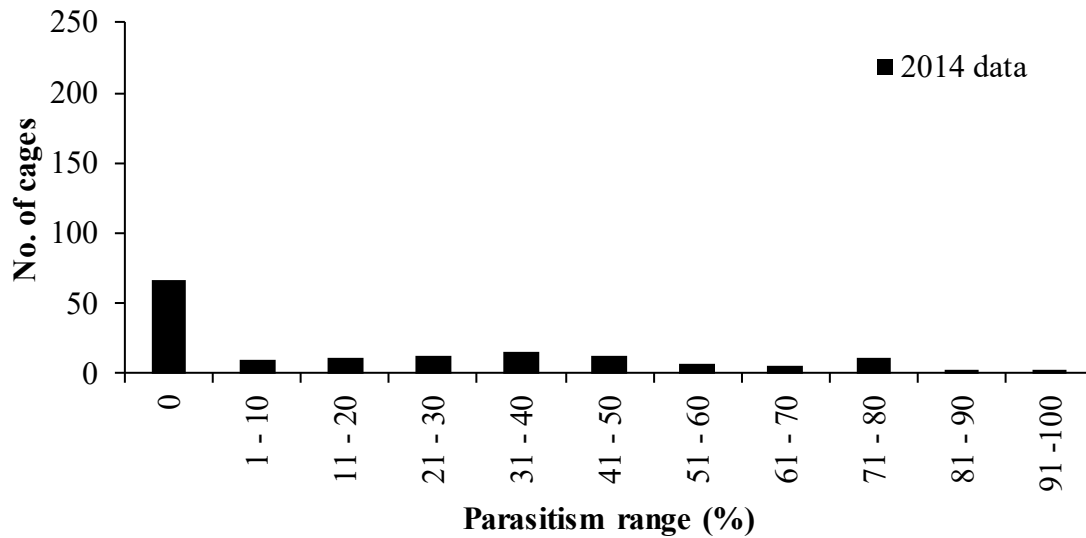


Figure 3.8. Frequency of parasitism at the Linwood depot in 2014, indicating the number of cages within each percentage parasitism class

The Linwood depot represents the area that had the highest tree mortality, which has since been reduced to pre-*S. noctilio* levels. Therefore, the depot trends and percentage parasitism are good benchmarks for all other depots with the variations recorded in **Appendix 9.1**.

The frequency of male and female *S. noctilio* emergence at the Linwood depot in 2007 (**Figure 3.9**) indicated that the mode for both sexes was in the 1-10 wasps per cage category. The same trend occurred in the 2014 data for the Linwood depot (**Figure 3.10**). Trends relating to the 2008 to 2013 emergence cage data are included in **Appendix 9.2**. Although most cages produced 1-10 wasps in both 2007 and 2014, the number of wasps dropped from 128 males and 319 females per cage in 2007 to 78 males and 108 females per cage in 2014, indicating a huge reduction in the *S. noctilio* population. This demonstrated the success of the inoculations of *D. siricidicola* and release of *I. leucospoides* (see later) to control the *S. noctilio* population. Since 2007, there has been a gradual increase in parasitism by *D. siricidicola*, with the highest levels observed in the 2014 data (**Appendix 9.2**).

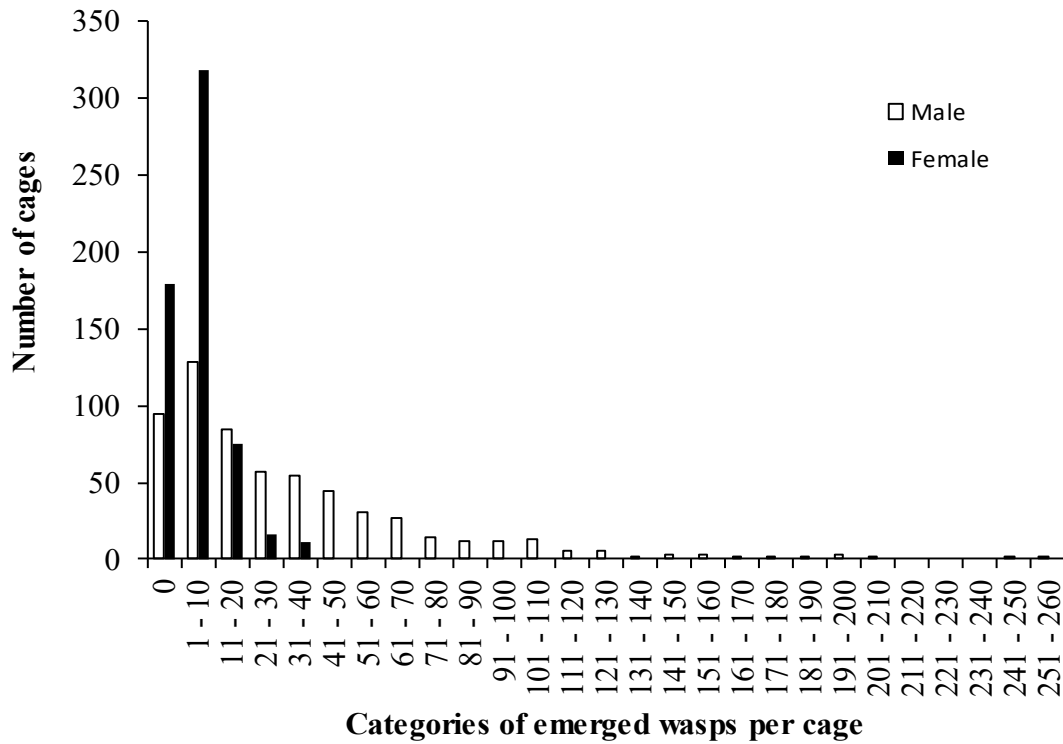


Figure 3.9. Frequency of *Sirex noctilio* emergence per cage at the Linwood depot in 2007.

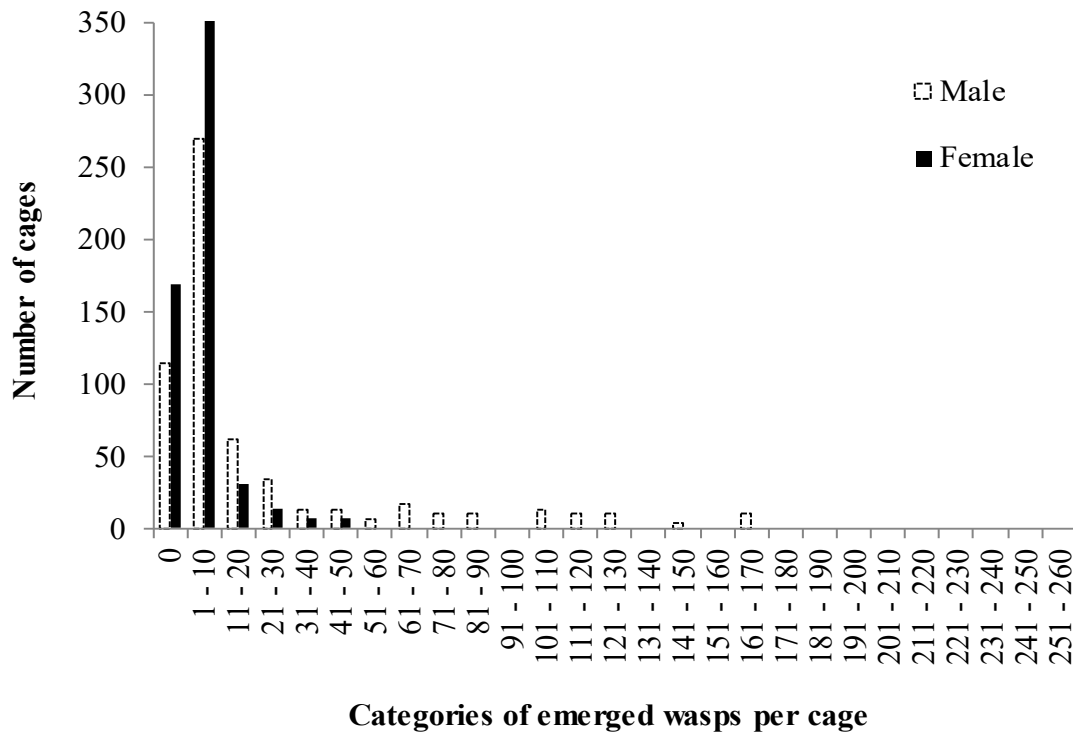


Figure 3.10. Frequency of *Sirex noctilio* emergence per cage at the Linwood depot in 2014.

3.1.3.3. Variations in depot parasitism results with *D. siricidicola*

Inoculations of *D. siricidicola* started in 2006 as part of the Industry Trial that had two sites in KwaZulu-Natal province. The main thrust to control *S. noctilio* in KwaZulu-Natal was initiated in 2007 with the start of mass inoculations. The numbers of nematodes that were requested from the culture facilities for inoculation by the SASCP, the actual numbers that were received and inoculated, as well as the numbers of trees that were planned for inoculation and that were inoculated during the eight-year inoculation period are indicated in **Table 3.7**.

The *D. siricidicola* utilised in all inoculations were from the same source and were cultured at FABI. A standard operating procedure ensured that the handling and inoculation process was similar in all regions and only four contractors with small teams were used. Therefore, any variations in results were due to the climatic variation across the country and the population size of *S. noctilio*.

Initial inoculations with *D. siricidicola* produced very low levels of parasitism. The 2006 Industry Trial tested certain assumptions to determine the reason for poor levels of parasitism with the trial results and indicated that the best time for inoculation with *D. siricidicola* was mid-February to May. Based on these results, the 2007 mass inoculation was undertaken. The first inoculations generally provided 5% parasitism which increased to 12% after the second inoculation a year later. The third inoculation generally produced around 20% parasitism, which increased thereafter due to natural distribution of the nematodes by *S. noctilio*.

The area serviced by the Linwood depot was inoculated annually until 2010. There was a steady increase in parasitism from 2007 to 2010 (**Table 3.8**). The initial results were high due to the mass inoculation distributing high numbers of *D. siricidicola* into the population of *S. noctilio*. In 2007, the plan was to inoculate 91 000 trees of which 57 000 trees were inoculated in KwaZulu-Natal (**Table 3.7**).

The Vryheid depot was not impacted by a mass inoculation and the volume of nematodes inoculated was reduced due to the tree search and inoculation initiative in an area where *S. noctilio* was still becoming established. The less intensive release of *D. siricidicola* resulted in the lower levels of parasitism experienced by the depot in 2011 and 2012 (**Table 3.8**). The areas that were infested with *S. noctilio* were increasing northwards into areas

Table 3.7. Number of trees inoculated in the Southern Cape (SC), Eastern Cape (EC), KwaZulu-Natal (KZN), Mpumalanga (MP) and Limpopo (LM) provinces between 2007 and 2014

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014
Supplier	FABI	Australia	FABI	FABI	FABI	FABI	FABI	FABI	FABI
Nematodes									
Planned	8 629 760 000	2 000 000 000	800 000 000	487 000 000	1 080 000 000	1 000 001 000	990 000 000	765 600 000	
Actual	Trial 4 641 301 000	3 115 000 000	830 000 000	478 400 000	1 007 700 000	977 400 000	980 000 000	865 000	
% Delivered		53.8	155.8	103.8	98.2	93.3	97.7	99.0	113.0
Trees inoculated									
Planned		90 982	20 000	8 000	5 020	10 800	10 100	9 809	8 836
Actual		57 205	31 150	8 300	4 672	10 762	9 682	9 387	8 810
% Inoculated		63.0	192.0	104.0	93.0	99.6	99.6	95.7	99.7
Areas inoculated	KZN	EC, KZN	EC, KZN	EC, KZN	SC, EC, KZN, MP	SC, EC, KZN, MP	SC, KZN, MP, LM	SC, MP, LM	EC, MP, LM

where initial inoculations were carried out. Second and third year inoculations then increased the parasitism rate.

Table 3.8. Wasp emergence per log at the three depots in South Africa and the percentage of wasps that were parasitized by *D. siricidicola*

Depot	Year	No. of emerged wasps		No. of logs	Average wasps per log		Parasitism %	
		Male	Female		Male	Female	Male	Female
Linwood	2007	19907	3078	1727	11.5	1.8	16	19
	2008	10237	1991	1715	6.0	1.2	20	33
	2009	9763	1652	1116	8.7	1.5	18	26
	2010	4491	757	585	7.7	1.3	49	66
	2011	2397	880	459	5.2	1.9	53	55
	2012	1929	794	396	4.9	2.0	53	48
	2013	3303	599	423	7.8	1.4	41	50
	2014	3821	812	492	7.8	1.7	31	36
George	2010	5914	1608	369	16.0	4.4	28	26
	2011	5442	1203	312	17.4	3.9	25	19
	2012	3103	798	294	10.6	2.7	30	25
	2013	2611	810	273	9.6	3.0	37	29
	2014	2286	590	324	7.1	1.8	31	27
Vryheid	2010	4901	871	300	16.3	2.9	16	25
	2011	15199	2774	714	21.3	3.9	7	7
	2012	19867	3047	975	20.4	3.1	8	7
	2013	22831	2634	1267	18.0	2.1	15	14
	2014	15267	2125	1212	12.6	1.8	26	22

The areas serviced by the George depot have had *S. noctilio* present for many years and while *D. siricidicola* was parasitizing *S. noctilio*, the impact of *I. leucospoides* (discussed later) was very evident. These areas have had very few inoculations carried out during 2007 – 2014 (Table 3.9) and parasitism has been maintained by natural distribution of *D. siricidicola* by *S. noctilio*.

3.1.3.4. Inoculation programme size

The number of trees to be inoculated was driven by several factors, namely poor parasitism results from the previous year, the number of *S. noctilio*-affected trees with low or no parasite (*D. siricidicola* or *I. leucospoides*) impact and the potential economic loss. This inoculation cost was tempered against available resources, considering that the cost of *D. siricidicola* and field staff to release *D. siricidicola* was approximately at a 50:50 ratio. The cost of *D. siricidicola* (per 100 000 nematodes) in 2007 was R 52.00, compared to R 46.00 in 2008 and R 68.00 in 2014, creating an expenditure of approximately R 15 000 000.00. The numbers of inoculated trees by DAFF region, apart from trial work carried out between 2004 and 2006, are recorded in **Table 3.9**.

The timber industry had a management guideline (Verleur *et al.*, 2016) which stipulated that *S. noctilio*-related tree mortality should be less than 5% and parasitism should be over 20%. The 2014 emergence cage parasitism results indicate that the parasitism threshold was exceeded (**Figure 3.6**).

Table 3.9. Number of trees inoculated, by DAFF region, in a commercial manner, to reduce the tree mortality to economically acceptable levels

Province	No. of trees inoculated annually								Total
	2007	2008	2009	2010	2011	2012	2013	2014	
Southern Cape				1176	708	426	100	200	2610
Eastern Cape	2666	500	1100	1010	850				6126
KwaZulu-Natal	54539	32045	5600	1281	151	300	186	0	94102
Mpumalanga				1205	9053	7853	6797	7256	32204
Limpopo						63	504	141	708
Swaziland						1000	1800	350	3150
Total	57205	32545	6700	4672	10762	9682	9387	7947	138900

Parasitism of *S. noctilio* by *D. siricidicola* is a vital monitoring aspect and should the parasitism drop off due to management operations, such as harvesting, (removing the mature tree habitat necessary to support *S. noctilio* and *D. siricidicola* populations) then parasitism

levels will indicate the need for further inoculation to prevent outbreaks of *S. noctilio* tree damage.

The number of wasps per log that emerged at the Linwood depot in 2007 ranged from 0 to 85.3 for males and 0 to 13.3 for females. The average numbers of male and female wasps per log was 11.5 and 1.8, respectively (**Table 3.8**). The number of wasps emerging per log at each of the three depots generally decreased from when the depots were initially set up to when the data were last recorded in 2014 (**Table 3.8**). However, wasp emergence varied between the depots, or the regions that they represented.

Linwood, the first depot to be established, which reflected the outcomes of the mass inoculation campaign in 2007, and the subsequent follow-up inoculations in 2008, had by 2011 recorded the lowest numbers of emerged wasps per log (**Table 3.8; Figure 3.11**). In comparison, higher numbers of wasps per log were recorded at the George depot. In this region, many plantations have been returned to natural flora and fynbos, resulting in reduced areas planted under *P. radiata*, as well as harvested areas replanted to *P. elliottii*, a species that is less preferred by *S. noctilio*. Inoculations in this area have been successful, but the background parasitism level remains low. The Vryheid depot indicated a large *S. noctilio* population by the higher numbers of emerged wasps per log (**Table 3.8**). This was also verified by the National Monitoring Initiative results (**Chapter 5**), which showed that in these areas there were many dying trees, together with high numbers of wasps emerging per log with a lower parasitism rate.

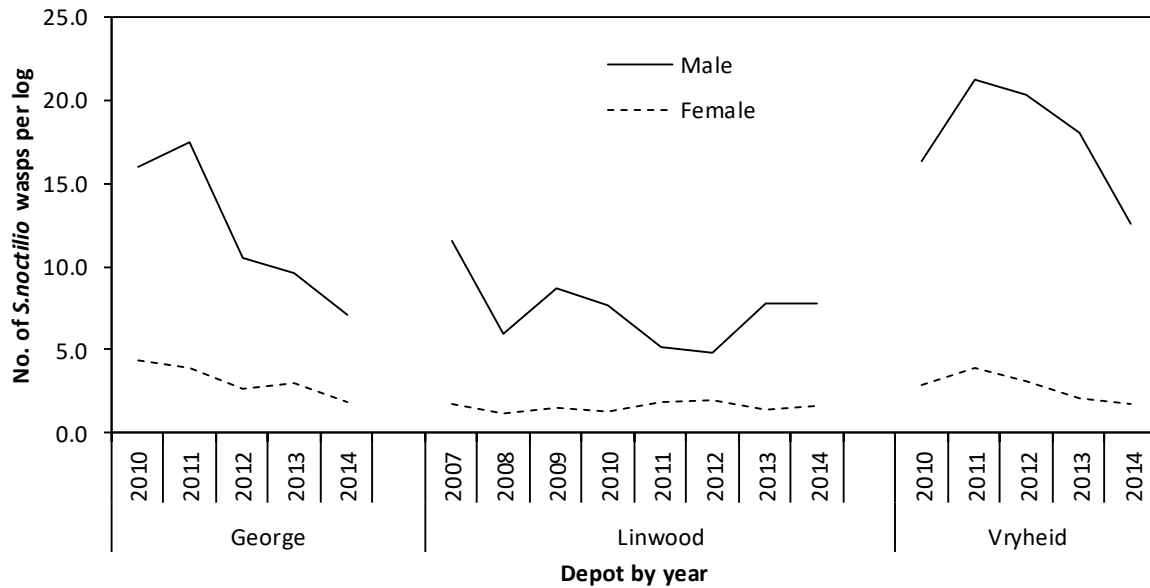


Figure 3.11. Wasp emergence per log over time at the three depots in South Africa.

During 2007 to 2014, the wasps emerged from the second week of October until the third week of December. This emergence pattern was related to the increase in moisture and temperature within the logs (which causes the fungus to die and triggers pupation and wasp emergence) that were loaded into the cages (Madden, 1988). This pattern was also reflected in the results from the panel traps, which caught *S. noctilio* females (see **Chapter 4**).

Emergence patterns are shown in **Figure 3.12**, indicating the annual fluctuations, with higher emergence in certain weeks taking place, specifically in 2011, 2012 and 2013 due to sunny weeks resulting in more males of *S. noctilio* emerging than during rainy colder weeks. Week one of emergence is week 41 on the annual calendar, which is usually the second week in October. The overall emergence patterns at the Linwood depot had been annually consistent (**Figure 3.13**) in that during each year the wasps emerge within the same week. **Figure 3.13** indicates that the emergence of male *S. noctilio* peaks a week earlier than in the female wasps.

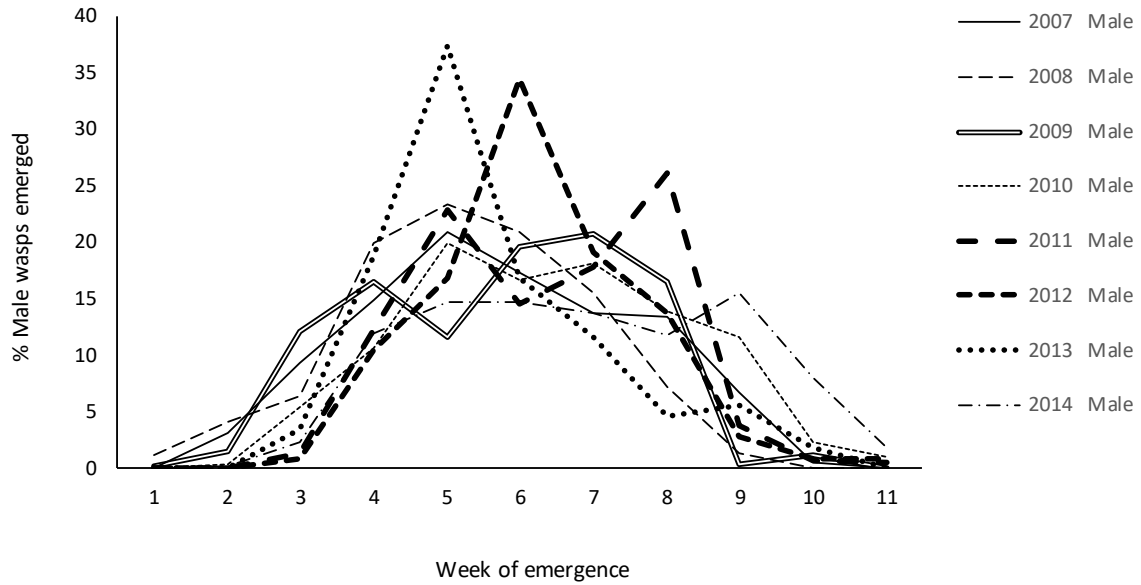


Figure 3.12. Weekly emergence of *Sirex noctilio* males between 2007 and 2014 as a percentage of the total number of emerged males at the Linwood depot.

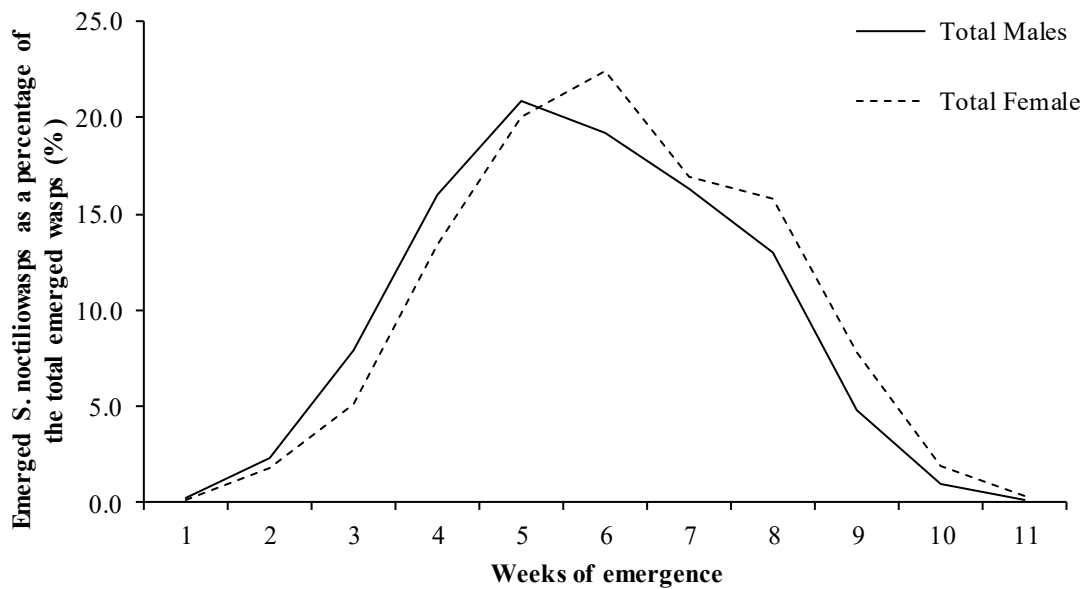


Figure 3.13. Average weekly emergence of male and female *Sirex noctilio* at the Linwood depot from 2007 to 2014.

3.1.3.5. Comparing monitoring results

The environmental conditions at each emergence cage depot, such as light and temperature, may have influenced the start and duration of *S. noctilio* emergence, compared to the plantation environment. Each depot was different in construction, with the George depot making use of an old school building. Although there was a roof and ceiling, the windows and doors were kept open allowing the free movement of air. In contrast, the Linwood and Vryheid depots comprised old milking sheds with corrugated iron roofs (no ceiling) and no restriction on ventilation.

To determine if the emergence cages influenced wasp emergence during an emergence season, wasp emergence from the emergence cages at Linwood depot was compared with emerged wasps captured in panel traps (**Chapter 4**) that were suspended in plantations in KwaZulu-Natal. The number of wasps that emerged per week during the 2007 emergence period in the emergence cages were compared to those caught in the panel traps during the same time. The numbers of wasps that emerged or were caught per week were shown as a percentage of the total number of wasps that emerged or were captured (**Figure 3.14**).

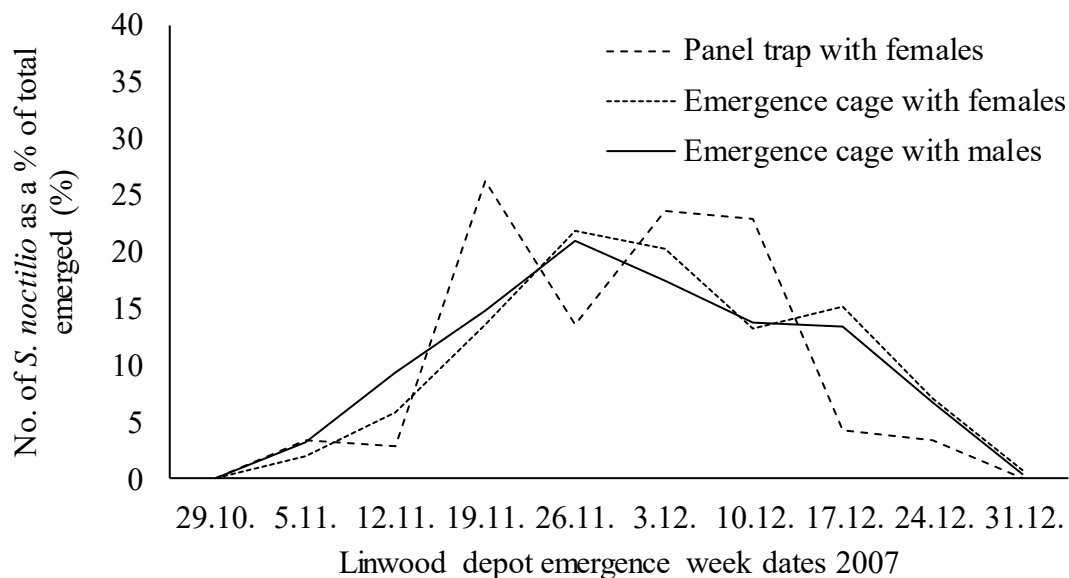


Figure 3.14. Comparison of *S. noctilio* emergence as determined by the emergence cages and panel traps in KwaZulu-Natal in 2007

Although there was a slight deviation between the data from the traps and emergence cages, the periods of peak female emergence largely coincided (**Figure 3.14**). These slight deviations may be due to variation in temperature, which may fluctuate less in the depot than in the field.

3.1.4. Conclusions and recommendations

3.1.4.1. Future sampling efforts

It has not been possible to reduce the numbers of wasps that need to be dissected to confirm parasitism by *D. siricidicola* for the following reasons:

- The percentage parasitism increased from the first until the last emergence of the wasps. Therefore, all wasps from the initial and final emergence needed to be assessed.
- During the first weeks of emergence, mainly male wasps and few female wasps emerged, while later in the emergence period the number of emerging females increased.
- Wasp size also seems to change over the emergence period, with larger females emerging later in the emergence period.
- The total number of wasps that could emerge, within the emergence cages at the depots, at either a regional or individual cage level, cannot be predicted, which prevents a more sophisticated sampling process from being implemented.

The following sampling strategy is proposed to achieve a more accurate measure of *D. siricidicola* parasitism at an area level, based on a small sample at a compartment level, and still reduce the number of wasps required for dissection. This strategy will consider the following aspects:

- Trap tree plots that were established during the previous year and inoculated in the current year should be represented in emergence cages by trees that have evidence of *S. noctilio* larvae in the sample logs. These trees would have been inoculated with *D. siricidicola* and should therefore indicate a high level of parasitism, being a measure of both inoculated and background parasitism.

- Sites to be sampled should be ranked according to their climatic risk, which is calculated at an industry level, with sub-level categories such as silviculture management of either sawtimber or pulpwood regime, poor or no history of parasitism, and areas where the knowledge of parasitism of either *D. siricidicola* or *I. leucospoides* is lacking. Sites that are geographically close together and under the same regime could be combined.
- Areas which were inoculated but do not have established trap tree plots should be sampled to ensure that *D. siricidicola* was effectively inoculated and remained effective in its infective form to complete the parasitic function.
- Areas not sampled above which would constitute a risk to pine growers, should include small growers, or farm and plantation areas, which were previously harvested and now enter the 10-year age class when *S. noctilio* could re-infest the growing area, with no known biological control releases.

3.1.4.2. Inoculation planning and considerations

Each year, the number of trees inoculated by the SASCP was determined by the previous year's emergence cage parasitism results. Where more than 10% of the *S. noctilio* wasps were parasitized by *D. siricidicola* (Bedding, 2009), the background parasitism from previous years' inoculations, together with the current year's inoculation parasitism, should increase the parasitism so that further intervention is not required. Therefore, the emergence cages must provide information that distinguishes between parasitism in inoculated trees versus uninoculated trees. In many instances, sample logs were collected from inoculated trees to ensure that the inoculations were successful, and to determine the level of success that was achieved. It was also beneficial to collect logs from the same compartment to determine the background parasitism and thus whether inoculations from previous years were successful.

After several years, it was not possible to differentiate between the background and inoculated parasitism, but the overall parasitism level remained the critical measure in maintaining and reducing the *S. noctilio* population to acceptable levels. These measures should be linked to plantations, farms or compartments so that any plantation, farm or compartment below the 10% parasitism level can be identified and re-inoculated.

Since pine plantations in South Africa are fragmented across the landscape, this may hinder the spread of *D. siricidicola* from farm to farm. This fragmented landscape may also be beneficial as it can slow the movement of pests, since there is no homogenous land use. As discussed above, sampling based on the number of living trees, or trees per ha, or per ha per age class, or per number of *S. noctilio*-infested trees, would need far more cages than is feasible. Smaller samples were collected from numerous farms, which gave a reasonably accurate indication of parasitism for the region. Predictions could then be made regarding individual farms that were not sampled, based on the plantations, farms or compartments that had received similar inoculation treatments near the then un-sampled farm or compartment. Initially, the sample logs were collected from farms where inoculations had taken place, while the background samples were taken from nearby compartments to determine the effectiveness of the inoculations. Inoculations on a plantation, farm or compartment were terminated once high parasitism was confirmed, or *S. noctilio*-infested trees, from which to collect the sample logs, could not be located. At this point, monitoring for *S. noctilio* damage needed to rely on the National Monitoring Initiative (**Chapter 5**) or the creation of trap trees to monitor the *S. noctilio* population development and trends in tree damage.

3.2. IBALIA LEUCOSPOIDES

3.2.1. Introduction

3.2.1.1. *Ibalia leucospoides* background

The parasitoid wasp, *I. leucospoides*, is native to Europe and lays its eggs within the eggs of *S. noctilio*. After hatching, the larvae live as endoparasitoids of *S. noctilio* larvae (Zondag, 1959). Female *I. leucospoides* locate the oviposition channels created by *S. noctilio* females through the scent of the *A. areolatum* fungus and oviposit directly into the egg or newly hatched *S. noctilio* larvae (Tribe and Cillie, 2004). The emerging parasitoid larvae consume the *S. noctilio* larvae, thereafter pupating and emerging from the tree as adults. The wasp is able to parasitize up to 40% of the *S. noctilio* population (Fernandez-Arhex and Corley, 2005).

In South Africa, the initial introduction of *I. leucospoides*, imported from Uruguay in 1998 by the Plant Protection Research Institute (PPRI), consisted of only 18 males and 19 females (Tribe and Cillie, 2004). The PPRI eventually released 456 individuals in the Western Cape between 1998 and 2001 (Tribe and Cillie, 2004), which established and spread throughout *S. noctilio* populations. From this first introduction as well as the distribution of logs containing *S. noctilio* larvae and *I. leucospoides* throughout the Western and Southern Cape, the population of *I. leucospoides* has increased. According to (Tribe and Cillie, 2004), *I. leucospoides* is well adapted to a Mediterranean climate and a cold dry habitat rather than temperate zones (Spradbery and Kirk, 1978). This established population was utilised by the SASCP in their catch and release programme that actively spread *I. leucospoides* throughout South Africa. The SASCP contracted the PPRI to collect *I. leucospoides* for release in KwaZulu-Natal during the late 2005 and early 2006 flight season. Thereafter, the SASCP erected their own cages, filled them with *S. noctilio*-infested logs, and recovered *I. leucospoides* from these logs.

Since *I. leucospoides* larvae utilize a single host, there is a one-to-one relationship between *S. noctilio* larvae and *I. leucospoides*. This allows easy calculation of *I. leucospoides* parasitism levels by dividing the numbers of *I. leucospoides* by the total numbers of *I. leucospoides* plus *S. noctilio* that emerged in any given volume of timber. The fecundity of

I. leucospoides is beneficial for control since the parasitoid can build a larger population than *S. noctilio* in a shorter period. *Sirex noctilio* has a skewed sex ratio ranging from 5-32 males for every female, with the average female depositing around 250 eggs (Iede *et al.*, 1998). In contrast, *I. leucospoides* displays an equal sex ratio (1.15 males to 1 female), with the average female depositing around 600 eggs (Tribe and Cillie, 2004).

Annually, since 2007, the SASCP have erected cages to collect *I. leucospoides* and these have yielded various favourable outcomes. Without destroying the log, it is not possible to visually detect whether *I. leucospoides* are present or absent in a log. *Sirex noctilio* tell-tale signs were more easily visible and if *I. leucospoides* has been released in the area, then logs collected from those areas should produce the parasitoid.

3.2.1.2. Aims of the catch and release programme

The aim of the *I. leucospoides* catch and release strategy was to increase the distribution and natural dispersal rate of *I. leucospoides* throughout the pine-growing areas of South Africa. However, the production of large numbers of *I. leucospoides* in a laboratory was more complex than the rearing of *D. siricidicola*.

3.2.2. Materials and Methods

3.2.2.1 Rearing and releases

For successful rearing of *I. leucospoides* in large numbers, logs with numerous newly-laid *S. noctilio* eggs or first-instar larvae are required, into which the mated females will oviposit (Zondag, 1959). This process of providing oviposition sites continues until sufficient *I. leucospoides* adults have emerged for transport to the release sites.

The timing of the release of newly-emerged *I. leucospoides* must be synchronised with the availability of newly-laid eggs or first-instar larvae of *S. noctilio* in the region of release. The two distinct rainfall regions in South Africa create a staggered *S. noctilio* emergence pattern, which affects the matching of the parasitoid-host life cycles. Where large numbers of *I. leucospoides* are available, a catch and release approach removed the need to maintain a complicated breeding programme.

In the winter rainfall regions of the Western, Southern and Eastern Cape, *I. leucospoides* emerges two weeks after the *S. noctilio* emergence period, which is from mid-November to the end of April. In the summer rainfall regions of KwaZulu-Natal, Mpumalanga and Limpopo province, *S. noctilio* emerges from the last week of October to mid-December with *I. leucospoides* emerging two weeks later. There was an abundance of *I. leucospoides* in the Western and Southern Cape region where most *I. leucospoides* had been caught, but due to the shorter *S. noctilio* emergence period in the KwaZulu-Natal, Mpumalanga South, Mpumalanga North and Limpopo regions, only *I. leucospoides* that emerged from November to January could be released in these regions. *Ibalia leucospoides* that emerged in February to May in the Southern Cape region needed to be released back into this region, since the *S. noctilio* in the KwaZulu-Natal, Mpumalanga South, Mpumalanga North and Limpopo regions were already beyond the second instar stage of development. Mature larvae would have penetrated too deep into the timber to be located by the female *I. leucospoides*.

3.2.2.2. *Ibalia leucospoides* parasitism

The levels of parasitism by *I. leucospoides* in pine plantations in South Africa were measured by three methods, which were differentiated by the volume of wood being tested. The first method recorded parasitism at the level of the individual emergence cage from which *I. leucospoides* and *S. noctilio* emerged. The second method combined these cage totals and calculated parasitism for the depot. The first method was more beneficial in that the origin of the logs in each cage was known and that, despite the small log sample, the presence of *I. leucospoides* indicated successful establishment of a large population. This information was used to focus releases on plantations and farms where *I. leucospoides* was absent and thereby increase the dispersal of the parasitoid.

The third method involved stacking *S. noctilio*-infested logs (remaining portions of trees felled when collecting sample logs) in large insectaria and then counting the emerging *I. leucospoides* and *S. noctilio* to determine the percentage parasitism. This method was good for collecting large numbers of *I. leucospoides* for release, as the logs originated from previous *I. leucospoides* release sites or from areas that supported large populations of *I. leucospoides*. However, this parasitism calculation does not reflect an area or regional scenario as logs from various areas were often combined to fill the large insectaria and ensure the harvesting of many *I. leucospoides*.

Although a few *I. leucospoides* were released in KwaZulu-Natal as early as 2005, it was only in 2010 when the population had grown to such an extent that *I. leucospoides* were captured from *S. noctilio*-infested logs in emergence cages in KwaZulu-Natal.

3.2.2.3. Dispersal of *Ibalia leucospoides*

The SASCP adopted the catch and release approach for the release of *I. leucospoides* to coincide with the movement of *S. noctilio* northwards through the country, as determined by the panel trap monitoring (Chapter 4). Table 3.10 indicates by province the regions where *I. leucospoides* has been released.

Table 3.10. The year and province where adults of *Ibalia leucospoides* were captured and released.

Year of capture	Place of capture	Quantity captured	Province of release
2005/6	Western Cape	177	KZN, E. Cape
2006/7	W. Cape, KZN	417	KZN
2007/8	W. Cape, KZN	276	KZN, Mpumalanga
2008/9	W. Cape, KZN	536	KZN, Mpumalanga
2009/10	S. Cape	2189	E. Cape, Mpumalanga
2010/11	W. + S. Cape, KZN	4349	E. Cape, KZN, Mpumalanga
2011/12	S. + E. Cape, KZN	6611	E. Cape, KZN, Mpumalanga, Limpopo
2012/13	S. Cape, KZN	3966	S. Cape, KZN, Mpumalanga, Swaziland, Limpopo, FABI (for breeding experiment)
2013/14	S. Cape, KZN	3301	S. Cape, E. Cape, Mpumalanga, Limpopo
2014/15	S. Cape, KZN	2844	E. Cape, KZN, Mpumalanga, Limpopo
Total		24666	

3.2.3. Results and Discussion

3.2.3.1. Successful release areas

Ibalia leucospoides was captured in varying numbers in the Southern, Eastern and Western Cape regions and released in KwaZulu-Natal and later in Mpumalanga and Limpopo provinces (Table 3.10). The place of capture, except for the Western Cape, shows areas where *I. leucospoides* was successfully released into plantations since logs from those areas were collected, and *I. leucospoides* were recovered from these logs. This indicated that

successful mating, oviposition, pupation and emergence had occurred in the summer rainfall regions of South Africa. Initially the trapping programme, which tracked the spread of *S. noctilio* northwards through South Africa, indicated new areas which *S. noctilio* had invaded. It was in these areas where *I. leucospoides* was released. Later when the trapping programme was no longer required, areas of poor *D. siricidicola* parasitism were chosen as sites to release *I. leucospoides*. Presently, the individual companies request where *I. leucospoides* should be released.

The level of parasitism was determined from the numbers of *S. noctilio* and *I. leucospoides* that were reared from the emergence cages (**Table 3.11.a**) and the insectaria (**Table 3.11.b**) at the three depots, with Linwood receiving timber from 2 additional sources. The regions in which *I. leucospoides* was captured, confirmed that populations have become established in those regions. The parasitism level was also calculated for each depot and insectarium since 2006 where the number of *S. noctilio* that emerged, were counted.

In **Table 3.11a**, the George depot received logs from areas where the *I. leucospoides* population had been established for over 10 years; hence the consistent parasitism results from 2010 to 2014. Linwood and Vryheid were areas where *I. leucospoides* has no recorded historical presence and the parasitism results show an increase in parasitism from 0% to 40.5% for the KwaZulu-Natal area, represented in the Linwood depot. Vryheid was slower to build *I. leucospoides* parasitism, since *S. noctilio* entered the area later than KwaZulu-Natal and the area under forestry is much larger. Both Linwood FABI (timber from a FABI trial) and Linwood Lothair represent timber from an area usually represented in the Vryheid depot. Therefore, the parasitism results are in line with Vryheid depot results. In **Table 3.11b**, the logs that were collected and placed in the insectaria were from areas where *I. leucospoides* had been previously released. The aim was to collect as many *I. leucospoides* as possible for redistribution to areas where *I. leucospoides* parasitism was low or where no previous releases had taken place. The parasitism percentage indicates the successful establishment of *I. leucospoides* in the areas of log collection.

Table 3.11a. *Ibalia leucospoides* parasitism at the various depots where wasp emergence was recorded in emergence cages.

Depot	Year	Total emerged <i>S. noctilio</i>			Cages	% Parasitism
		Male	Female	Total		
George		19356	5009	24365	3789	13.5
	2010	5914	1608	7522	638	7.8
	2011	5442	1203	6645	1412	17.5
	2012	3103	798	3901	893	18.6
	2013	2611	810	3421	525	13.3
	2014	2286	590	2876	321	10.0
Linwood		53031	10328	63359	4080	6.0
	2007	19907	3078	22985		0.0
	2008	10237	1991	12228	4	0.0
	2009	9763	1652	11415		0.0
	2010	4491	757	5248	100	1.9
	2011	2397	880	3277	354	9.7
	2012	1929	794	2723	1282	32.0
	2013	3303	599	3902	1264	24.5
	2014	1004	577	1581	1076	40.5
Vryheid		78065	11451	89516	775	0.9
	2010	4901	871	5772	0	0.0
	2011	15199	2774	17973	19	0.1
	2012	19867	3047	22914	18	0.1
	2013	22831	2634	25465	68	0.3
	2014	15267	2125	17392	670	3.7
Linwood FABI		16976	1761	18737	298	1.6
	2013	9864	627	10491	2	0.0
	2014	7112	1134	8246	296	3.5
Linwood Lothair	2014	2817	235	3052	3	0.1
Total		170245	28784	199029	8945	4.3

Table 3.11b. *Ibalia leucospoides* parasitism at the various depots where wasp emergence was recorded in insectaria designed to catch large numbers of *I. leucospoides* for release.

Depot	Year	Total emerged <i>S. noctilio</i>			Insectaria	% Parasitism
		Male	Female	Total		
PPRI	2005				177	
George		27438	91685	36606	14002	1.2
	2006			No count	41	
	2007			No count	75	
	2008	680	203	883	442	33.4
	2009	6928	2599	9527	2189	18.7
	2010			No count	3374	
	2011	11466	3024	14490	4630	24.2
	2012	4496	1481	5977	1791	23.1
	2013	2988	1345	4333	1199	21.7
	2014	880	516	1396	261	15.8
Tweedie					602	
	2006			No count	376	
	2007			No count	201	
	2011			No count	25	
Linwood		270	100	370	430	53.8
	2008			No count		
	2010	263	93	356	237	40.0
	2011			No count	20	
	2013			No count	93	
	2014			No count	80	
Vryheid		1107	79	1186	359	23.2
	2008			No count	90	
	2012			No count	0	
	2013			No count	152	
	2014	1107	79	1186	117	9.0
Ugie					151	
	2011				151	
Total		28815	9347	38162	15721	29.0

3.2.3.2. Considerations concerning a catch and release approach

For biological control of *S. noctilio* by *I. leucospoides*, two management aspects must be considered. The first was the possible impact of insectivorous birds, as was the case in Tasmania (Madden, 1982), which prey on *I. leucospoides*. Despite this threat, *I. leucospoides* has become established in the Eastern Cape, KwaZulu-Natal and Mpumalanga provinces.

The second aspect is a consideration of the high cost of inoculating with *D. siricidicola* relative to the cheaper option of collecting *S. noctilio*-infested logs and harvesting the emerged *I. leucospoides* adults for releases in areas where parasitoid populations are low or absent. The catch and release of *I. leucospoides* has proved to be a successful method to harvest large numbers of *I. leucospoides*, with over 24 000 *I. leucospoides* adults released over the eight-year period of the SASCP (2007 – 2014).

3.2.3.3. Future requirements from the depots

The depots need to be operational to monitor parasitism levels and ensure that they are sustained through inoculations of *D. siricidicola* and confirmation of the presence of *I. leucospoides* in ever-changing forestry age class, species and product cycles.

Ideally, every farm should be monitored but provided that all the climatically high-risk areas and the more vulnerable pine species are monitored regularly, an outbreak could be averted.

This annual parasitism verification remains vital in preventing *S. noctilio* outbreaks in the future, and will offer continued success if reasonable samples are brought in from areas of high climatic risk and vulnerable timber stocks.

4.0. RECORDING THE MIGRATION OF *SIREX NOCTILIO* THROUGH SOUTH AFRICA USING PANEL TRAPS

4.1. Introduction

The accidental introduction of *Sirex noctilio* in Cape Town in 1994 (Tribe and Cillie, 2004) initiated the migration of this pest through South Africa over subsequent years. *Sirex noctilio* moved slowly through the Western Cape Province and southern part of the Eastern Cape Province. In 2002, *S. noctilio* had entered the northern part of the Eastern Cape and southern KwaZulu-Natal, causing tree mortality to increase at alarming rates as well as the establishment of a large pest population in the summer rainfall areas of South Africa. The Western Cape Province and the southern Cape region (winter rainfall area) supported plantations of pine species that originated from Europe and Asia, whereas the northern Eastern Cape, KwaZulu-Natal, Mpumalanga and Limpopo Provinces supported pines that originated from Mexico and North America (Hinze, 1993; Meyer, 2009). *Sirex noctilio* had therefore crossed over onto pine species that were not endemic to its natural distribution, as well as into areas with a seasonal rainfall variation (Hurley *et al.*, 2007). This increased the risk of tree mortality for the remainder of South Africa north of KwaZulu-Natal Province. A trap network was thus implemented to determine the presence of *S. noctilio* in areas where it had not previously been recorded.

The trap network, which incorporated kairomone lures in panel traps, was implemented in 2007 and annually thereafter until 2014. The results of this programme assisted in the deployment of biological control where *S. noctilio* was detected in the traps (positive traps), and follow up assessments quantified the number of infested trees. This information was presented to the Forestry Industry landowners on an annual basis so that they could manage the threat with the immediate deployment of biological control.

The aim of monitoring the movement of *S. noctilio* through the remaining pine-growing areas of South Africa was to detect the wasp's presence and then release *D. siricidicola* and *I. leucospoides* as soon as it was detected. The provinces where *S. noctilio* had not been recorded, prior to 2007, included northern KwaZulu-Natal, Mpumalanga and Limpopo, which had extensive areas planted to pine for sawtimber and pulpwood production. Traps were therefore deployed in these areas to monitor the full extent of *S. noctilio* migration and

attempt to prevent the development of large populations of *S. noctilio*. On many occasions, following the detection of *S. noctilio* in traps, inspection of the surrounding plantation trees revealed no evidence of tree mortality. Thus, the traps were more effective than stressed trees in attracting female *S. noctilio* wasps. A ‘positive trap’ site refers to a site with three traps where one or more traps caught *S. noctilio* females, and on the odd occasion a male wasp. A ‘negative trap’ site refers to a site where none of the three traps captured any *S. noctilio* wasps.

In 2010, the *S. noctilio* climatic risk mapping model was developed (**Chapter 6**), and was available for application to many facets of the SASCP. In 2011, two criteria were used to select trap sites, namely pine compartments that were 10 to 20 years of age and had a high climatic risk rating. These areas were further north of the migration range, as it was in 2011, so the full extent of the migration of *S. noctilio* could be measured.

4.2. Materials and Methods

Black panel traps with a pherolure (manufactured by Insect Science (Pty) Ltd, Tzaneen, South Africa) that attracted female *S. noctilio* were used in this study. The compound in the pherolure was a blend of (+)- α -pinene (12.5%), (-)- α -pinene (12.5%), (-)- β -pinene (25.0%), (+)-3-carene (30.0%), (+)-camphene (5.0%), β -myrcene (10.0%), (+)-limonene (2.5%) and (-)-limonene (2.5%) (Hurley *et al.*, 2015). A sponge (approximately 4 x 6 x 2 cm) was soaked in the mixture and placed in a plastic bag, which was attached to the trap. The lure was not specific to *S. noctilio* but attracted any insect that was attracted to stressed pine trees as the mixture simulated α and β -terpenes that are emitted by stressed pine trees (Simpson, 1976).

The efficacy of the panel trap in attracting female wasps with this specific kairomone lure was first tested in 2007 in the midlands of KwaZulu-Natal Province, where the presence of *S. noctilio* was evident. This panel trap study to test the efficacy of the lure, referred to as the lure trial, involved 11 sites around two locations, one outside Greytown at Seven Oaks and the other outside Howick, with three panel traps placed per site (**Table 4.1**).

Table 4.1. Panel trap trial site locations indicating the number of *Sirex noctilio* wasps that were trapped in the corresponding positive trap inspections.

Site No.	Nearest Town	Position	Female <i>S.noctilio</i> caught	No. of inspections with no <i>S. noctilio</i>	Trap rendered ineffective *	Inspections when <i>S. noctilio</i> found	Trap found on the ground
55	Howick	Open Area	72	14	0	13	0
56	Howick	Open Area	42	13	0	13	1
57	Howick	Plantation Pine	52	12	0	15	0
58	Howick	Plantation Pine	62	14	0	13	0
59	Howick	Open Area	21	12	3	10	2
60	Seven Oaks	Euc compartment	64	7	0	20	0
61	Seven Oaks	Euc compartment	47	8	0	19	0
62	Seven Oaks	Plantation Pine	31	12	0	15	0
63	Seven Oaks	Plantation Pine	26	15	0	12	0
64	Seven Oaks	Timber depot LD 24	1	26	0	1	0
65	Seven Oaks	Timber depot LC 18	2	24	0	2	1
			420	157	3	133	4

* = Trap ineffective through theft, bucket had fallen off, etc

During the *S. noctilio* flight season of 2007, traps with the *Sirex* kairomone lure were set up in areas where the presence of *S. noctilio* was known (KwaZulu-Natal Midlands) and in areas to the north of KwaZulu-Natal where *S. noctilio* was likely to have spread. In 2007, traps were set up south of Howick in KwaZulu-Natal where *S. noctilio* was present, and northwards as far as Lydenburg in Mpumalanga Province, to measure the full extent of *S. noctilio* migration. The urgency in determining the full extent of the spread of *S. noctilio* was to identify areas for the release of *D. siricidicola* early in 2008. In 2008, the traps were deployed across the northern KwaZulu-Natal region, and in Mpumalanga and Limpopo Provinces.

Trap site selection was based on observations in pine pulpwood plantations, which showed that *S. noctilio* had started to affect trees from 10 years of age upwards in densely planted compartments (3 m x 2 m). Where a wider planting density (3 m x 3 m) was implemented, *S. noctilio* started affecting trees from 12 years of age and upwards. Initially, traps were set up at convenient and accessible places in pine plantations, especially of *P. patula*, and of trees older than 10 years, for 10 weekly inspections. This initial approach worked well from 2007 to 2010. From 2011 to 2014, the trapping sites were determined by means of the *S. noctilio* climatic risk model.

Trap placement within the compartment was usually inside the compartment boundary on the first tree line from the road. Where theft was a potential problem, the panel traps were placed deeper into the compartment. On several occasions, traps were placed in Eucalyptus compartments adjacent to pine compartments and these traps were successful in luring *S. noctilio*. Three traps were placed at each trap site and labelled as A, B or C with the trap site number. Traps were placed approximately 100 m apart and secured to a tree or trap bracket so that the lure was 2 m above the ground. Trees with branches did not require brackets, but sawtimber trees pruned above 3 m in height required brackets to secure the traps. This allowed the trap to hang vertically, permitting *S. noctilio* females to enter the trap, slide down the trap sides and land in the collecting bucket. At sites where silviculture operations such as thinning and pruning were expected, the traps were placed outside the compartment boundary in open grassland areas using a stand, from which the trap was hung. **Figure 4.1** illustrates the three methods used for securing traps in or near the trap site.

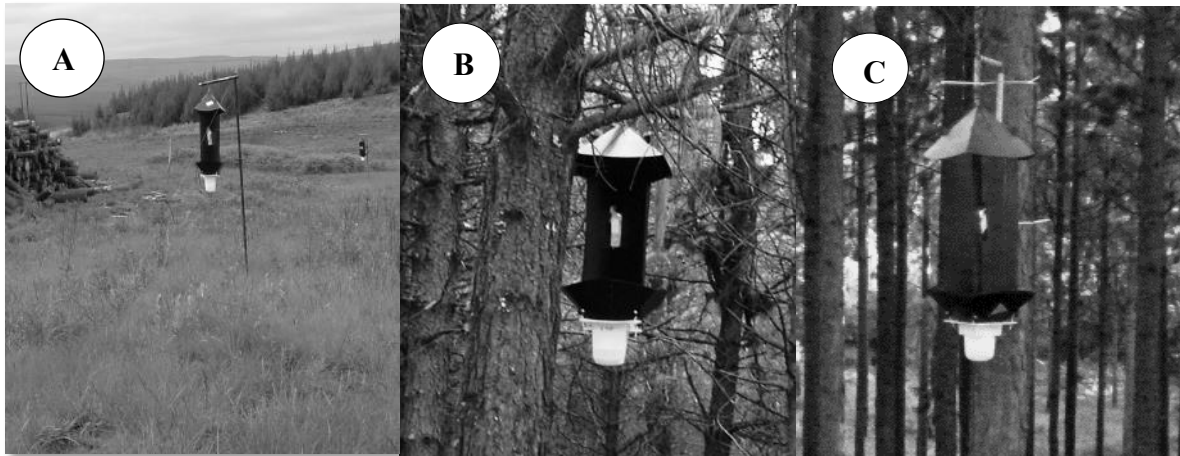


Figure 4.1 Placement of panel traps on a stand in an open area adjacent to a timber stack (A), on a branch in a compartment (B) and on a pruned tree using a bracket (C).

The panel traps were inspected weekly to collect the insects trapped in the buckets, and record the presence or absence of *S. noctilio*. All captured insects were collected, and preserved in 70% ethanol. Insects besides *S. noctilio* were sent to FABI for identification. Records of *S. noctilio* presence were maintained for subsequent *D. siricidicola* and *I. leucospoides* releases.

The weekly inspection of the panel traps allowed for maintenance of the traps. This included the removal of debris and the replenishment of water in the trap bucket, or the removal of water following rain. A small amount of liquid soap was mixed into the water to break the surface tension, causing the insects to drown and thus preventing them from escaping. Regular inspection of panel traps was also necessary since they were sometimes tampered with by inquisitive people, knocked down by antelope or damaged by wind.

4.3. Results and Discussion

During the trap lure trial undertaken in 2007, 45% of the 297 weekly inspections revealed wasp catches (**Table 4.1**). The remaining 157 inspections showed no *S. noctilio* presence while four inspections recorded traps on the ground and three inspections revealed trap theft.

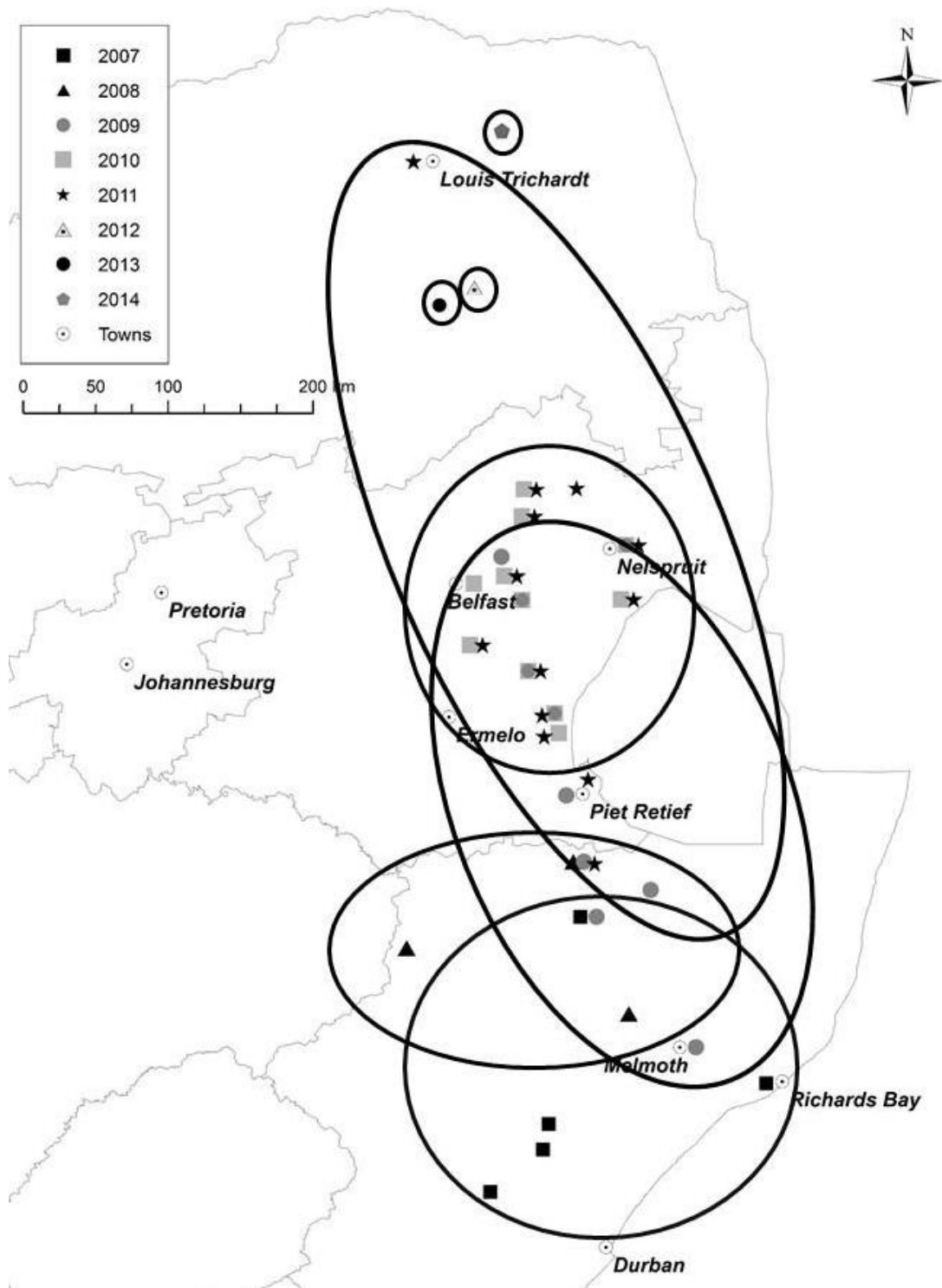


Figure 4.2. Spread of *Sirex noctilio* across South Africa as determined by the panel traps monitored from 2007 to 2014. Dispersal is grouped by year which illustrated years when the wasp spread over long distances or remained in a localised area. For geographic reference, the panel trap locations have been grouped according to the closest town as indicated on the map.

The geographical spread of *S. noctilio* as detected by the panel traps (**Figure 4.2**) illustrates the years in which *S. noctilio* remained locally, with no evidence of dispersal, either by natural migration or via timber on vehicles. In other years, such as 2009 and 2011, *S. noctilio* dispersed northwards, presumably by transport of timber, as the populations of *S. noctilio* increased and moved longer distances across South Africa.

The number of wasps caught by the traps (**Table 4.2**) in the applicable year of trapping is indicative of positive trap sites. The table grouped the trap sites according to the town closest to the trap sites, with the towns placed in directional order from south to north. This trap initiative excluded areas where *S. noctilio* had become established during the initial migration from 1994 to 2006 and focussed on areas which were colonised after 2006, or where *S. noctilio* populations increased in size in pine plantations.

Table 4.2. The number of female wasps trapped per year, with towns in South Africa ordered from south to north.

Towns	2007	2008	2009	2010	2011	2012	2013	2014	Total
Tsitsikamma			1						1
George			5	5					10
Hankey				7					7
Howick	249								249
Seven Oaks	171								171
Greytown	6								6
Richards Bay	1								1
Melmoth			12						12
Babanango		14							14
Normandene		6							6
Louwsberg			10						10
Vryheid	13	18	3						34
Paulpietersburg		8	10		4				22
Piet Retief			1		2				3
Amsterdam				1	12				13
Lothair			4	2	10				16
Warburton			2	16	83				101
Carolina				1	18				19
Badplaas			2	38					40
Barberton				4	9				13
Waterval-boven				2	1				3
Belfast				15					15
Machadodorp			1						1
Ngodwana				17	25				42
Nelspruit			2	1	7				10
White River									0
Lydenburg				2	15				17
Sabie					12				12
Haenertsburg							21		21
Tzaneen						2			2
Louis Trichardt					9				9
Thohoyandou								120	120
Total	440	46	53	111	207	2	21	120	1000

Blank spaces in **Table 4.2** indicate that either no traps were deployed or no wasps were caught. **Table 4.2** also indicates that *S. noctilio* was recovered in Tsitsikamma and George in the Southern Cape in 2009 and 2010. This is according to the trap network that started in 2007 and traps were used in this instance in the Southern Cape to enable the detection of

S. noctilio presence within that region. This was not the first recording of *S. noctilio* in Tsitsikamma and George as it was present in these areas in 2001 (Tribe and Cillie, 2004). The abundance of *I. leucospoides* and *D. siricidicola* in the Southern Cape (Tribe and Cillie, 2004) made it difficult to find *S. noctilio*-infested trees, as the *S. noctilio*-affected trees were dispersed over large areas because of the reduced *S. noctilio* population. The traps assisted in finding areas where *S. noctilio* was present and searches were then made around positive trap sites. This was undertaken to determine the status of *D. siricidicola* in the area so that logs could be collected for parasitism verification in the emergence cages.

In 2007, the trap sites ranged from Howick, KwaZulu-Natal to Graskop, Mpumalanga Province, but positive traps were located between Howick and Vryheid in northern KwaZulu-Natal (**Table 4.2**). In 2008, the trap site network was expanded northwards to Louis Trichardt, Limpopo Province, but positive traps indicated that the new range into which *S. noctilio* had spread was from Babanango, Zululand region to Paulpietersburg in northern KwaZulu-Natal. In 2009, the trap network was again expanded to the Southern Cape Region where *S. noctilio* was found in traps from Tsitsikamma, Southern Cape region northwards to Nelspruit in Mpumalanga (**Table 4.2**). During 2010, the same regions as in 2009 were served by trap sites with *S. noctilio* invading, for the first time, the Lydenburg area of Mpumalanga Province. In 2011, traps were deployed between Paulpietersburg, northern KwaZulu-Natal Province and Louis Trichardt, Limpopo Province with positive traps being recorded at Sabie, Mpumalanga Province and Louis Trichardt, Limpopo Province. During 2012, 2013 and 2014, traps were deployed between Sabie and Louis Trichardt (**Table 4.2**), but the number of trap sites was reduced (**Table 4.3**), since the presence of *S. noctilio* had been recorded throughout South Africa except for a few farms where these trap sites were established.

Table 4.4 indicates the numbers of positive and negative trap sites (i.e. those that did (+ve) and those that did not (-ve) catch *S. noctilio* females) during the 2007 trap season together with the numbers of *S. noctilio* females captured.

Table 4.3. Summary of the number of panel traps used annually with the percentage of positive traps increasing over the years, as the placement of traps improved through the climatic risk model.

Year	No. of Traps	No. of Panel Trap sites	No. of Positive trap sites	% Positive	Regions covered by traps	Comments
2007	195	65	17	26.2	KZN to Mpumalanga	Testing of traps in known <i>S. noctilio</i> areas
2008	435	145	11	7.6	KZN to Limpopo	Traps in accessible pine areas
2009	402	134	18	13.4	KZN to Limpopo	Traps in accessible pine areas
2010	285	95	24	25.3	KZN to Limpopo	Traps in accessible pine areas
2011	318	106	48	45.3	KZN to Limpopo	Traps placed in high risk sites
2012	15	5	1	20.0	Mpumalanga to Limpopo	Traps placed in high risk sites
2013	36	12	6	50.0	Limpopo	Areas where <i>S. noctilio</i> is undetected
2014	15	5	4	80.0	Limpopo	Areas where <i>S. noctilio</i> is undetected
	1701	567	129			

Table 4.4. The number of positive and negative trap sites during the 2007 trap season, together with the number of *Sirex noctilio* females captured.

	No. of Trap sites		No. of Wasps
	Negative	Positive	
2007	48	17	440
Howick		5	249
Seven Oaks		6	171
Greytown	1	1	6
Zululand Coast		1	1
Melmoth	4		
Richards Bay	1		
Helpmekaar	1		
Normandene	2		
Vryheid	1	4	13
Piet Retief	3		
Swaziland	3		
Panbult	1		
Amsterdam	2		
Lothair	1		
Warburton	3		
Carolina	1		
Badplaas	1		
Barberton	6		
Waterval-boven	1		
Belfast	1		
Machadodorp	1		
Ngodwana	2		
Lydenburg	2		
Sabie	9		
Graskop	1		

From **Table 4.4** it was evident that the Howick area had the highest captures of *S. noctilio* wasps, indicating the success of the kairomone lure used in the lure-testing trial to attract *S. noctilio* females. The start of the trapping programme also indicated that *S. noctilio* was present at Seven Oaks, Greytown, Vryheid and on the Zululand Coast in 2007. The Zululand Coast site was a few kilometres from the Richards Bay pulpwood stockpile for the paper-producing mill. The wasp was found in the closest pine sawtimber compartment but no other pine trees in the area showed signs of tree mortality due to *A. areolatum* and the phytotoxic mucus. There was also no evidence of the presence of *S. noctilio* in this area from 2007 to

2014, which is not surprising considering the higher temperatures along the coast in this region. This points to timber transport being a major mode of dispersal in South Africa.

In 2008, *S. noctilio* colonised the area around Vryheid and only moved slightly northwards during this flight period (**Table 4.2**). This area was comprised of predominantly pine sawtimber, but within this larger area, *S. noctilio* became established in compartments where thinning had been delayed. In 2008, the panel traps recorded *S. noctilio* as having reached the towns of Vryheid and Paulpietersburg, while the areas further north still showed no indication of the presence of *S. noctilio* (**Table 4.2**).

In 2008, the northern-most area where *S. noctilio* had been detected was Paulpietersburg, but in 2009, this extended to Nelspruit. This spread of 218 km (direct distance) suggests that the transport of timber products was the most likely cause of this rapid expansion. (Bruzzone *et al.*, 2009) demonstrated that a female *S. noctilio* wasp can fly a maximum of 49 km on a flight mill device, while (Villacide and Corley, 2008) indicated shorter maximum distances.

In 2010, the movement of *S. noctilio* continued from Nelspruit northwards to Lydenburg (67 km) and westwards to Belfast (97 km) with the areas in-between Nelspruit and Paulpietersburg being infested. The shorter dispersal distances suggested that 2010 was a colonisation period (**Table 4.2**). In 2011, *S. noctilio* moved northwards from Lydenburg to Louis Trichardt, covering 233 km in direct flight. Again, timber movement was likely to have assisted this movement of *S. noctilio*, for reasons previously discussed (**Table 4.2**).

In 2012, limited dispersal took place with positive signs of the wasp recorded at Tzaneen which lies between Nelspruit and Louis Trichardt. Although no wasps were trapped close to Sabie (small town of Graskop), there were some trees with evidence of oviposition by *S. noctilio* females (**Table 4.2**). In 2013, colonisation took place again and *S. noctilio* was found at Haenertsburg, near Tzaneen (**Table 4.2**). The traps set up in 2014 showed that the movement of *S. noctilio* was from Louis Trichardt and 25 km eastwards towards Thohoyandou (**Table 4.2**). Movement of *S. noctilio* to the warmer Thohoyandou was slow, but the wasp has since become established in pine sawtimber compartments in this area.

4.3.1. Panel trap assessments according to the climatic risk model for *Sirex noctilio*

The number of positive traps increased when the panel traps were placed in high-risk areas determined by the climatic risk model, within areas where *S. noctilio* was still undetected.

The climatic risk model (discussed in **Chapter 6**) was developed and utilised from 2011 onwards to assist in the selection of high-risk sites for trap placement. Trap placement was analysed under three climatic risk classes, namely high, intermediate and low. **Table 4.5** shows the number of traps allocated under each *S. noctilio* climatic risk category, including the years prior to the development of the model (2007-2010). The panel trap sites spanning all years were assigned to the three risk categories to confirm that the model was accurate across the South African pine-growing areas, and not just in the test area. Low risk areas were also selected to ensure that large pine plantation areas were not excluded in the monitoring. The decrease in the number of traps and trap sites over subsequent years was due to the decreasing area of undetected *S. noctilio*.

Table 4.5. Numbers of panel trap sites analysed according to the climatic risk model over the assessment period from 2007 to 2014.

Category of risk	2007	2008	2009	2010	2011	2012	2013	2014	Total
High		58	50	29	39	1	3		180
Intermediate		69	60	56	62	3	8		258
Low		14	10	9	9	1			43
Unknown risk	65		11				1	15	92
Total Traps	65	141	131	94	110	5	12	15	573

Unknown risk refers to sites where GPS points were not established and therefore the climatic risk category could not be determined.

Table 4.6 shows the percentage of trap sites per risk category that confirmed the presence of *S. noctilio*. The increase in the percentage of positive traps from 2010 to 2011 was due to the application of the climatic risk model. Although 2012 reflected a drop in the percentage of positive traps, the percentages for 2013 and 2014 were considerably higher (**Table 4.6**).

Table 4.6. Positive trap sites per climatic risk category as a percentage of the total number of panel traps.

Category of risk	2007	2008	2009	2010	2011	2012	2013	2014	Total
High		6.9	18.0	27.6	43.6	0.0	33.3		21.7
Intermediate		8.7	13.3	28.6	43.5	33.3	62.5		24.4
Low		7.1	0.0	11.1	44.4	0.0			14.0
Unknown risk	26.2		0.0				0.0	66.7	29.3
Overall	26.2	7.8	13.0	26.6	43.6	20.0	50.0	66.7	23.6

Unknown risk refers to sites where GPS points were not established and therefore the climatic risk category could not be determined.

The data showing the positive trap sites (as a percentage of the total number of traps) in the intermediate and low risk categories suggest that climatic risk was not the only factor that influences the spread of *S. noctilio* (total number of traps is shown in **Table 4.3**). Other factors such as tree moisture stress as well as the height of a tree within the canopy (light availability), and management regime (i.e. sawtimber or pulpwood rotation) all play a role in attracting or repelling *S. noctilio* female wasps (P. Croft, personal observation).

In South Africa, and especially in KwaZulu-Natal, the inoculation technique was adapted and developed in pulpwood regimes where nematode quantities per tree were standardised together with a technique to inoculate trees, while still standing. In this manner, numerous trees were inoculated with successful parasitism being achieved. Pulpwood plantations, due to the higher stocking levels created an environment conducive to small tree moisture stress. As soon as *S. noctilio* invaded the sawtimber plantations north of KwaZulu-Natal (2009, 2010, 2011), the inoculation strategy changed and inoculations of felled sawtimber trees with up to twice the number of *D. siricidicola* per tree, were required. Therefore, while this adaptation to the process was being refined for sawtimber plantations, *S. noctilio* populations increased. Therefore, the number of positive panel traps in sawtimber plantations, which were generally considered to be less attractive to *S. noctilio* than pulpwood plantations, was higher than expected (see **Table 4.7**), especially in the intermediate and low risk category.

Table 4.7. Numbers of positive panel trap sites by climatic risk and plantation product.

Category of risk	2007	2008	2009	2010	2011	2012	2013	2014	Total
High									
Pulpwood		3	9	4	7				23
Sawtimber		1		4	10		1		16
Intermediate									
Pulpwood		3	2	3	2				10
Sawtimber		3	6	13	25	1	5		53
Low									
Pulpwood					2				2
Sawtimber		1		1	2				4
Uncategorised									
Pulpwood	13								13
Sawtimber	4							10	14
Total	17	11	17	25	48	1	6	10	135

The data in **Table 4.7** indicate that in the high risk category, 59% of the positive trap sites were in pulpwood managed compartments, while 33% and 48% of the positive trap sites in the low and uncategorised risk categories, respectively, were in pulpwood compartments. The intermediate risk category had only 16% of the positive trap sites in pulpwood plantations. Better analysis of these data are indicated in **Table 4.8** where all the panel trap sites were indicated according to the risk category to which they were assigned, and the percentage of positive panel trap sites are indicated. By including all the panel trap sites, all the risk classes throughout South Africa in pine-growing areas, in the 10 year and older age class, were indicated and the variation in the risk class, between pulpwood and sawtimber could be determined.

Table 4.8. The total number of trap sites allocated to each climatic risk category and plantation regime, showing the number of positive panel traps as a percentage of total trap sites.

Category of risk	2007	2008	2009	2010	2011	2012	2013	2014	Total	% (+ve) traps
High										
Pulpwood		33	32	10	15				90	25.6
Sawtimber		25	18	19	24	1	3		90	17.8
Intermediate										
Pulpwood		23	16	11	6				56	17.9
Sawtimber		46	44	45	56	3	8		202	26.2
Low										
Pulpwood		5	4	3	4				16	12.5
Sawtimber		9	6	6	5	1			27	14.8
Uncategorised										
Pulpwood	33		3						36	36.1
Sawtimber	32		8				1	15	56	25.0
Total	65	141	131	94	110	5	12	15	573	23.6

It was in the high and uncategorised risk categories (i.e. 2007, before the climate risk model was developed) where the percentage of positive traps in pulpwood compartments exceeded that of sawtimber compartments (**Table 4.8**). In 2007, softwood in South Africa amounted to 440 400 ha of sawtimber and 225 429 ha of pulpwood, with almost twice as much sawtimber than pulpwood (Meyer, 2008). The sawtimber occurs mostly in Mpumalanga and Limpopo Provinces, where the panel traps were fully utilized. KwaZulu-Natal had predominately pulpwood compartments and was excluded in the analysis since the presence of *S. noctilio* was well established. Thus, the percentage of positive panel traps for the intermediate risk category in sawtimber compartments exceeds that in pulpwood (**Table 4.8**). The area under pulpwood or sawtimber compartment management thus influences the presence of *S. noctilio*.

4.4. Conclusions

The Vryheid sites were all pine sawtimber compartments in comparison to the Seven Oaks and Howick sites, which were pine pulpwood compartments and plantations. In the South African context, this was contradictory to expectations since pulpwood compartments are planted at higher densities, which leads to competition for light, nutrients and moisture. However, it was observed many times that trees under moisture stress attract *S. noctilio* in

preference to nearby sites, where the stocking density was high, and planted with a pine species that is preferred by *S. noctilio*. Thus, this indicates the success of the lure in attracting *S. noctilio* in sawtimber compartments.

Areas of planted pine surrounding positive panel trap sites (i.e. where traps caught *S. noctilio* females) were inspected for the presence of *S. noctilio*-infested trees. The panel trap data provided an early entry point to inoculate *S. noctilio*-infested trees, which were spread out throughout the compartments (as opposed to trap trees), long before field staff observed *S. noctilio* tree mortality. Field detection of new infestations was complicated in two ways. Firstly, field staff required visible tree mortality and this became evident only once many trees were infested and the *S. noctilio* population had increased in the area. Secondly, the panel traps indicated the presence of *S. noctilio* in the flight season in November and December in the summer rainfall areas of South Africa, whereas the first indication of larval infestation by the tree was mid-February. This awareness was only reported later during the inoculation period. The inoculation planning and nematode supply had to be finalised by December since *D. siricidicola* required an eight-week population growth period in flasks under laboratory conditions before inoculations could take place.

The abundance of *I. leucospoides* in the Southern Cape made (**Chapter 3**) it difficult to find *S. noctilio*-infested trees. The panel traps assisted in finding areas where *S. noctilio* was present and searches were then undertaken around positive traps to determine the status of *D. siricidicola* in the area so that management initiatives could be better implemented.

Due to the application of the *Sirex* climatic risk model, the number of positive trap sites increased to 45.3% in 2011 and decreased to 20 % in 2012. In 2013 and 2014, the number of positive traps increased to 50% and 80%, respectively. This increase in positive panel traps cannot only be attributed to the location of the trap sites based on the application of the climatic risk model. The available area in which *S. noctilio* had not been detected was becoming geographically smaller and the chances of finding *S. noctilio* had increased. The panel traps implemented in 2012 and 2013 were situated to detect *S. noctilio* in previously un-detected areas within the larger known *S. noctilio* migration area. Although trained SASCP contractors inspected plantation areas around the positive traps, trees containing *S. noctilio* larvae were often not located. This indicated the extreme efficiency of the kairomone lures in luring female *S. noctilio* into the traps and away from the surrounding stressed pine trees.

This panel trap monitoring was extremely useful in optimising panel trap placement and allowed for the immediate deployment of biological control where *S. noctilio* females were trapped. However, the presence and absence data recorded by the panel traps needed to be enhanced by a quantitative measure of plantation damage. Knowledge of where the pest occurred was beneficial in implementing biological control, although the numbers that needed to be released remained unknown due to the unknown tree mortality. A quantitative measure of tree mortality was required to calculate the costs of timber loss including the value-added aspect of processing plants. A quantitative approach also motivated for further inoculations of *D. siricidicola* and releases of *I. leucospoides*. Throughout the panel trap campaign, the volume of timber lost was obvious and quantification was not a necessity to obtain industry co-operation. The quantitative monitoring aspect is discussed in **Chapter 5** under the National Monitoring Initiative where tree mortality data were collected.

The panel traps were extremely beneficial in providing data to assist with the prevention of *S. noctilio* outbreaks that could have led to enormous losses. The panel trap study has provided the information for deployment of biological control agents thereby reducing excessive tree mortality, due to *S. noctilio*, north of KwaZulu-Natal in Mpumalanga and Limpopo provinces. The aims of monitoring the spread of *S. noctilio* and implementing early releases of *D. siricidicola* and *I. leucospoides* were thus achieved.

5.0. CLIMATE RISK MAPPING OF *SIREX NOCTILIO*

5.1. Introduction

With the spread of *S. noctilio* through KwaZulu-Natal, Mpumalanga and Limpopo during 2007 to 2014, the potential area of infestation increased substantially. It thus became necessary to prioritise the regions, districts and smaller areas where *S. noctilio* was most likely to become more destructive. This was especially important for the larger forestry companies that were managing extensive areas, as their ability to assess large areas over a short period was limited.

Ground surveys were necessary to ensure that the data collected and subsequent management decisions were based on good evidence. This also ensured that the contractors could successfully locate infested trees for inoculation with *D. siricidicola*. Sites for the release of *I. leucospoides* and for the National Monitoring Initiative (NMI) also needed to be representative of the status of *S. noctilio* in the vicinity. These requirements were addressed by risk mapping, which provided a tool to direct the SASCP to focus on areas that were more likely to be affected by *S. noctilio* and thus optimised the use of resources.

The objective in developing the climatic risk-mapping model was to predict the susceptibility of commercial pine plantations in South Africa to *S. noctilio* infestations (Ismail *et al.*, 2007).

5.2. Materials and Methods

The *S. noctilio* risk model was developed using the random forests (RF) classifier (Breiman, 2001), within the R statistical computing environment (R Development Core Team, 2014), following the methodology developed by Ismail *et al.*(2007). The random forests classifier assesses the relationships among a large number of predictor variables and a responsive variable. For the purpose of the *S. noctilio* risk model, bioclimatic variables were used as predictors and records of presence and absence of *S. noctilio* as the responsive variable. The model was applied to the South African pine plantations landscape where each map unit was assigned a score (0-1) representing the risk of *S. noctilio* outbreaks based on climate. Risk maps were developed in a Geographic Information System (GIS) environment to visually represent the risk of *S. noctilio* across the forestry landscape.

The resultant maps calculated the risk of *S. noctilio* infestation within the summer and winter rainfall regions of South Africa. The implemented model provided a tool for identifying various *S. noctilio* risk areas within the national forestry landscape (Germishuizen *et al.*, 2013). The model output assisted the SASCP by directing attention to areas where early detection of new infestations and outbreaks could have prevented severe tree mortality. Other operational activities, such as the selection of sites for the deployment of biological control agents and the placement of kairomone lure panel traps, were also enhanced.

The most important bioclimatic variables used in this model were mean annual evapo-transpiration, monthly evapo-transpiration and monthly rainfall. The common characteristics of the summer and winter rainfall regions were high levels of evapo-transpiration during the dry seasons, with subsequent rainy seasons, which induced physiological stress within the trees. Plantation variables such as silvicultural practices, spacing of trees, stand age and thinning regime, which also affected the trees' moisture stress and ultimately their attraction to *S. noctilio*, were not included in the model. The model output was converted into a risk score for each plantation compartment.

5.3. Results and Discussion

The results of the *S. noctilio* climatic risk model were presented in maps, giving a spatial representation of the results, and a database which allocated a numerical value between 0 (low risk) and 1 (high risk) for the risk rating. Apart from the long-term climatic data, the plantation databases were updated annually to produce *S. noctilio* risk maps using current compartment ages (10 to 20 years) to indicate the risk associated with the current crop. The *S. noctilio* risk gradient was calculated across South Africa (**Figure 5.1**), but also at an area level (**Figure 5.2**) and a farm level (**Figure 5.3**) with more detail visible on each of the farms or plantation compartments. The model could be run on the entire age class of a farm or company, which was useful when planning future species for planting with an understanding of the *S. noctilio* risk.

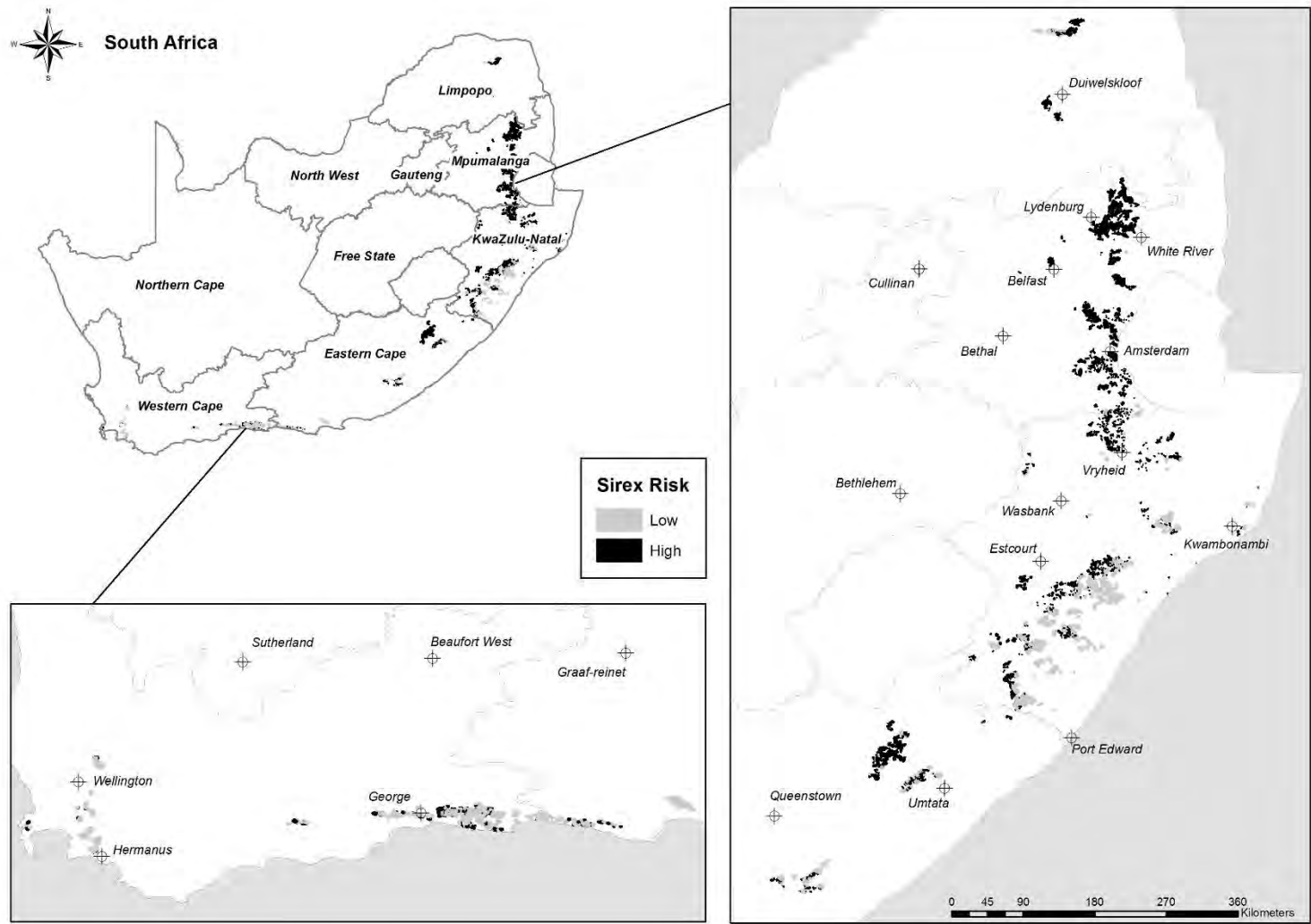


Figure 5.1. Climatic risk map for pine-growing areas in the winter and summer rainfall areas of South Africa, indicating areas at high and low risk of *S. noctilio* infestation.

5.3.1. Application to the panel trap programme

The application of the *S. noctilio* climate risk model to the panel trapping programme enabled the placement of traps in high risk areas as opposed to simply placing them in convenient places in the plantations. The panel traps that were set up from 2011 onwards (**Chapter 4.3.1**), indicated an improvement in trapping success when the traps were placed in high-risk areas.

5.3.2. Application to the National Monitoring Initiative

The NMI plot selection (**Chapter 6**) was randomly generated in the GIS system from data comprising the various large forestry companies' plantation databases. The *S. noctilio* climatic risk rating was then generated on the companies' plantation databases. The age classes of trees that are most susceptible to *S. noctilio* (10 - 20 year old compartments) were selected and the plot points were generated such that 10% of them were in low-risk areas and 90% of them in high and medium- to high-risk areas.

5.3.3. Application to the entire programme

The benefit of the *S. noctilio* climatic risk model to the SASCP was evident in several ways. The model output was firstly produced for the entire country (**Fig. 5.1**) providing a good indication of which areas in South Africa were more susceptible to *S. noctilio*. This allowed the SASCP to focus on the higher risk areas, and prioritise the pine pulpwood or sawtimber plantations, where necessary, as these plantation management strategies introduce a secondary risk at various levels. This provided an effective hierarchy across the country for monitoring, detection and assessment to determine the levels of tree mortality in the most critical areas. The model identified 75 000 ha of pine in the winter rainfall region where 78% was classified as low risk, 17% at high risk and 5% at moderate risk. In the summer rainfall region, the model identified 500 000 hectares of pine plantation, with 83% classified as moderately susceptible in the risk range of 30% to 60%. Only 3% of the plantation areas were classified as highly susceptible in the risk range of 60% to 100%, with the remaining 14 % classified as low risk in the range of < 30%.



Figure 5.2. Example of a company level map showing areas at high and low risk of *S. noctilio* infestation, as determined by the climatic risk model.

Another output from the model was at the forestry company level, to enable the individual companies to distinguish between the high- and medium-high risk sites on their land holdings. Each company usually produces either pulpwood or sawtimber, ensuring that the risk induced by their silviculture management is the same across all their farms. **Figure 5.2** presents a risk map from a company perspective, illustrating which plantations are at a high risk of *S. noctilio* infestations.

The model was also applied at a farm level in order to prioritise compartments within the farm and indicate where inspections for *S. noctilio* should be focussed (**Figure 5.3**).

Companies were also able to apply the risk rating model to make informed decisions on species choices for future planting.

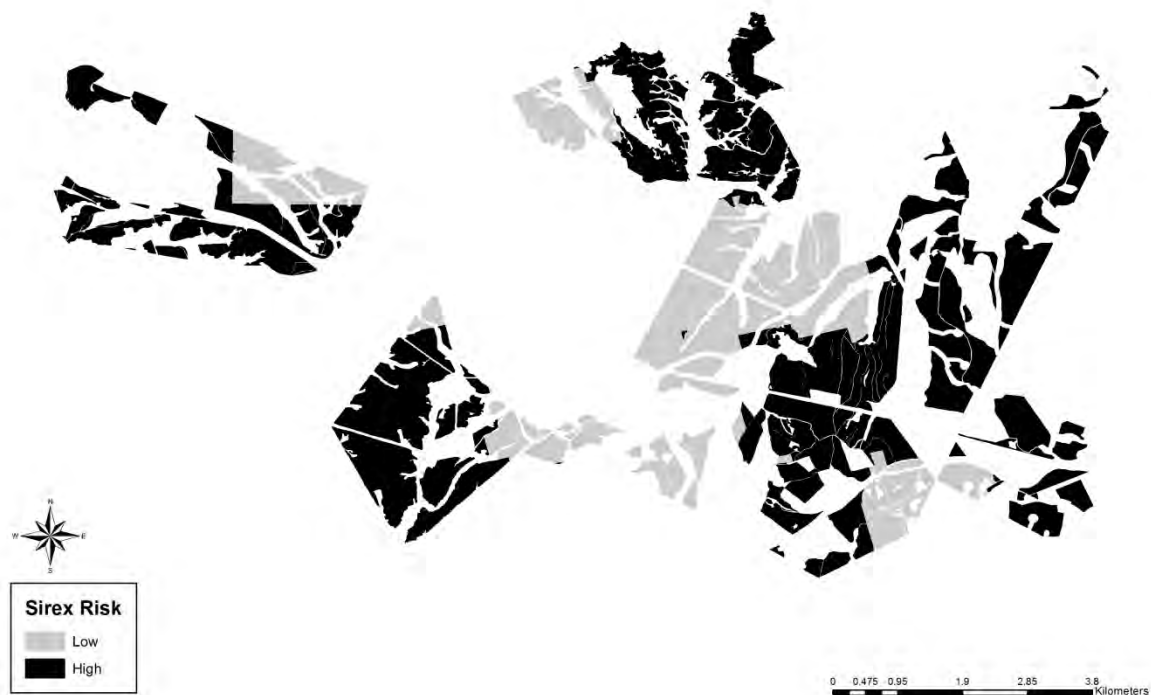


Figure 5.3. Example of a farm level map showing compartments at high and low risk of *S. noctilio* infestation, as determined by the climatic risk model.

5.4. Conclusion

The risk rating maps and databases were distributed to the respective forestry companies and small growers, who have used the output extensively. Some companies have used these resources to determine areas that required either trapping to determine the presence of *S. noctilio*, or parasitism verification through the placement of logs in emergence cages. These were also used extensively by the SASCP to determine the appropriate level of involvement by the companies based on their risk rating and for allocation of the NMI plots (**Chapter 6**). The ability to make better recommendations to companies and private growers has reduced expenditure for both companies and the SASCP. These recommendations have also achieved greater advantage by strategically placed biocontrol agent inoculations.

6.0. DEVELOPMENT OF THE *SIREX* NATIONAL MONITORING INITIATIVE

6.1. Introduction

The introduction of *S. noctilio* into South Africa in 1994 was recorded by the PPRI, who also monitored the rate at which *S. noctilio* spread in the Western Cape Province (Tribe and Cillie, 2004). In 2004, the South African *Sirex* Control Programme (SASCP) was formed and in 2007, a trapping programme was implemented using kairomone traps to monitor the movement of *S. noctilio* as it spread across South African forestry regions (Dyer *et al.*, 2007). The aim was early detection, which created an opportunity for the immediate release of the parasitic nematode, *D. siricidicola* (as discussed in **Chapter 4**). The trapping programme was implemented annually until 2011, with fewer traps used in 2012 to 2014 in Limpopo Province, since the pine-growing area where *S. noctilio* had remained un-detected was very limited. The reduction in traps was due to confirmation of the presence of *S. noctilio* in most areas, since the panel traps were confined to areas where *S. noctilio* had not previously been detected. Panel traps only indicated the presence or absence of *S. noctilio*, but were not suitable for determining the population density of *S. noctilio* in an area.

South African forestry plantation areas are fragmented across the eastern and southern regions of the country, due to land ownership, climate and topography. Within this national landscape, the areas are further fragmented and comprise three plantation types, namely species of *Acacia*, *Eucalyptus* and *Pinus*. The 10 large corporate companies, many smaller companies and private landowners all produce various timber products, which add increasing complexity when mapping the area and determining the age classes and species composition of the plantations. Forestry products in South Africa include sawtimber, pulpwood, poles, and a host of smaller industries. The movement of *S. noctilio* was regardless of land ownership, or products produced, and was assisted by timber transport. Communication with all landowners was necessary but no single channel of communication existed. The SASCP focussed on the large corporate companies and relied upon their willingness to co-operate with the initiatives of the SASCP. In this approach, most the pine-growing areas had achieved some level of control with *S. noctilio*, but the medium and small growers had not always participated or had the same exposure to the SASCP, although all timber producers were included in the programme. It was detrimental to the objectives of the SASCP to

achieve low *S. noctilio* tree mortality only on the large landowners' properties and not on those of the small growers due to a communication and awareness failure. The *Sirex* National Monitoring Initiative (NMI) was thus implemented to ensure that monitoring points were placed on small, medium and large growers' land, according to the climatic risk model results (see **Chapter 5**). This ensured that the areas at risk to infestation by *S. noctilio* were monitored annually and that appropriate action was taken where necessary to reduce the wasp population before high tree mortality was experienced.

Two levels of *S. noctilio* monitoring are recognised in South Africa, namely a national level under the NMI, and a landowner level. These levels are differentiated by the amount of detail / data collected.

The national level strategy had three focus areas:

- A broad annual quantification of *S. noctilio* damage per DAFF region to determine which areas need more attention to reduce *S. noctilio*-related tree mortality.
- Identification of areas where more intensive monitoring was required.
- Creation of a small-scale picture of *S. noctilio* infestation trends over space and time (Dyer *et al.*, 2007).

Monitoring at the landowner level was required to supply an annual quantification of *S. noctilio* damage at the farm level. There were several reasons for this level of detail, which included:

- Reporting of timber losses and adjustment of expected yields, which led to financial evaluation adjustments.
- Identification of compartments where *D. siricidicola* inoculations and *I. leucospoides* releases were required.
- Monitoring the success of these biocontrol operations as a driver for future operations.
- Monitoring where *S. noctilio* appeared to be in small undetectable numbers, but posed the potential to cause severe damage if not checked annually.
- Creation of a company perspective of *S. noctilio* infestations to support decision-making at the operational level and justification for further expenditure, where necessary.

- Adjustment of the harvest schedule in situations of excessive tree mortality, together with the development of a market for dry *S. noctilio*-infested timber.

Monitoring can be defined as a series of subsequent assessments on the status of an entity over time, which provides a process of detection of whether a change has taken place, as well as establishing the direction and extent of the change (Ferretti, 1997). The purpose of the *Sirex* NMI was to monitor, on an annual basis, tree mortality caused by *S. noctilio* across all the pine-growing areas in South Africa, and follow the trends, created by the annual results, with appropriate, cost effective biological control. Trends from the results would verify that the control measures were effective as well as assist in the development of norms. These norms would indicate at which level of tree mortality further inoculations were required, to reduce the threat in other areas. The aim was to facilitate early detection of outbreaks and identify where, and when, interventions were required.

In 2012, when the *S. noctilio* distribution range included all commercial pine plantations in South Africa, the use of baited panel traps (**Chapter 4**) was discontinued and the NMI was developed and implemented. The NMI was developed to meet the following objectives:

- To evaluate annually *S. noctilio* damage per geographical area.
- To identify areas where interventions were required (e.g. thinning, biological control inoculations).
- To compare spatial and temporal patterns of *S. noctilio* damage.

6.2. Materials and Methods

The *Sirex* NMI has continued since its initiation in 2012. The aim was to record tree mortality per hectare due to *S. noctilio* (Dyer *et al.*, 2012). National monitoring points were selected only where *S. noctilio* damage was expected, based on pine species, age class and climatic risk variables.

6.2.1. Selection of NMI sites

Site selection was determined by contacting the commercial pine plantation owners and requesting their plantation data in the form of shapefiles (i.e., files with geospatial vector data specifically for GIS programme applications).

Site selection was based on guidelines proposed by the FAO (Tomppo and Andersson, 2008) and used the Eurogrid Sampling design with a regular grid of detached transects (Germishuizen *et al.*, 2013). Pine plantations were stratified according to the climatic risk rating of *S. noctilio*, allowing 90% of the points in medium- to high-risk areas, and 10% of the points in low-risk areas. The low-risk points were included to detect if *S. noctilio* was present and ensure a full spectrum of presence and absence data throughout the country. Only compartments between the ages of 10 to 20 years were considered for site selection. Most timber growers submitted annual returns to DAFF containing the species, age class and purpose of the trees grown on their land. The total number of hectares under pine for the NMI was based on the data published by DAFF in 2009 (Meyer, 2009). This report was based on 12 regions within South Africa, which were designated by DAFF (Figure 6.1). It was not possible to allocate the location of the plantation timber data into the nine National Provinces since the data did not have a spatial component.

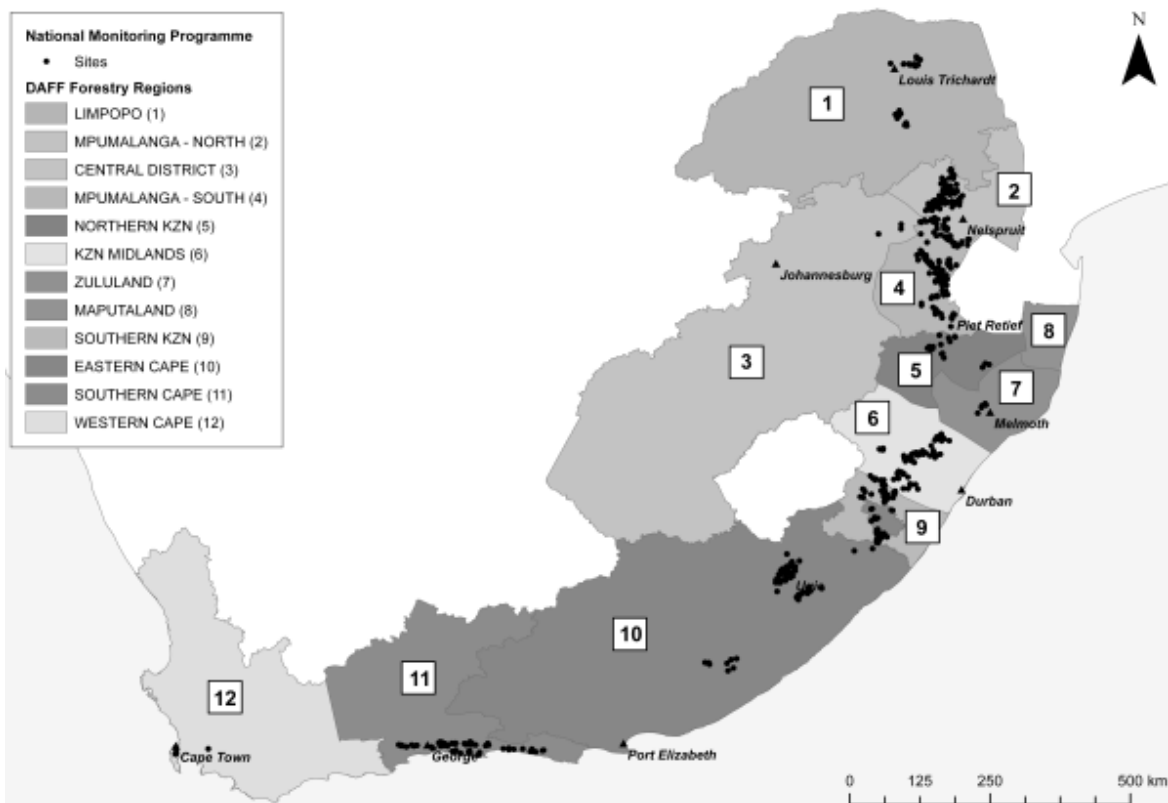


Figure 6.1. The 12 DAFF regions upon which the pine hectare data and NMI points results are presented.

In 2009, the total area under pine plantation in the DAFF regions was 675 000 ha of which 59% was sawtimber and 41% pulpwood. Of this, the total area of pine between 10 and 20 years of age was 324 000 hectares (**Table 6.1**).

Table 6.1. Regional distribution of pine of 10 to 20 years of age in South Africa in 2009.

DAFF region	Hectares	Proportion (%)	
		Sawtimber	Pulpwood
Limpopo	17 600	100	0
Mpumalanga North	78 800	87	13
Central District	11 000	18	82
Mpumalanga South	64 100	48	52
KZN Midlands	26 600	41	59
Northern KZN	10 100	50	50
Southern KZN	27 900	47	53
Eastern Cape	44 100	65	35
Maputaland	1 300	0	100
Zululand	3 200	93	7
Southern Cape	30 100	99	1
Western Cape	9 200	100	0
	324 000		

6.2.2. Determination of monitoring points

The EuroGrid Sampling Design (Tomppo and Andersson, 2008) determined that there should be one monitoring point for every 900 ha where dense monitoring is required, with one monitoring point every 200 ha in surveys requiring a sparse plot distribution (Tomppo and Andersson, 2008). Following this guideline, the SASCP required the establishment of 1615 NMI points following the sparse plot distribution.

6.2.2.1. Generation of permanent monitoring points

Of the 1615 NMI points, 90% were placed in *S. noctilio* medium- to high-risk areas (risk \geq 30%) and 10% in low-risk areas (risk \leq 30%). The cut-off between low-risk and medium- to high-risk was set at the 30% risk level because the *S. noctilio* Risk Model used only bioclimatic variables, which did not consider other risk factors such as silviculture practices, stand stress levels, occasional droughts, and fires etc. (**Chapter 5**). The number of points generated in the *S. noctilio* low-risk and medium- to high-risk category was 161 and 1454, respectively.

6.2.2.2. Determination of sample points

A *S. noctilio* risk map was generated for South Africa on which a 500 m x 500 m grid was overlaid. A centroid was generated for each grid square in the 500 m x 500 m grid (**Figure 6.2 A**). The 1615 points were randomly selected and one to nine transects, depending on tree density and method (see below), were established at each point. A centroid was also generated for each risk map feature (**Figure 6.2 B**) (Germishuizen *et al.*, 2013).



Figure 6.2. *Sirex noctilio* risk maps indicating examples of a centroid point for each grid square (A), a centroid for each risk map feature (B), a centroid closer to the midpoint that was linked (C) and the closest centroid point linked to create a base dataset (D).

Maps prepared by Dr I. Germishuizen (ICFR).

The *S. noctilio* risk map feature centroid, which was closest to each grid centroid, was selected to create the base dataset (**Figure 6.2 C**). **Figure 6.2 D** shows the final random point for assessment in the plantation compartments.

From this base dataset, 53 (10%) low-risk points and 482 (90%) medium- to high-risk points were randomly re-selected as indicated on **Figure 6.3**. These were used for the reduced point monitoring (see below).



Figure 6.3. Randomly generated NMI points showing their distribution throughout the pine-growing areas of South Africa.

The 1615 points covered 323 000 ha of pine in the 10 to 20-year old age class. At each point, a line transect was prepared and each tree was numbered to assist in subsequent years' assessments. Two sampling methods, namely individual point sampling and reduced point sampling, were considered since each has different benefits. The reduced sampling point method was implemented.

6.2.2.3. Reduced point sampling

Individual point sampling required extensive travelling and person-days, which increased the cost of monitoring. To reduce these costs, whilst retaining a broad base of sample points, the reduced point method was developed.

One third of the original 1615 points were randomly selected to provide 535 sites. At each site, three transects, in both pulpwood and sawtimber plantations, were established within walking distance of each other (100 m). The 535 sites included 53 points (10%) in low-risk areas and 482 points (90%) in medium- to high-risk areas. In thinned sawtimber compartments, additional transects were laid out to ensure that sufficient trees were assessed at each site (a minimum of 75 trees). To accomplish this in compartments where the thinning had reduced the stems to 250 trees ha⁻¹, up to nine transects were assessed. In pulpwood compartments, each point included three transects. All trees within these transects were assessed by the criteria in **Table 6.2**.

Table 6.2. Criteria used to assess the status of individual trees, in relation to *S. noctilio* infestation.

Tree status	Assessment criteria
Living	Green crown and no sign of <i>S. noctilio</i> damage,
Dead due to <i>S. noctilio</i>	Exit hole present from previous years' emergence,
Dying due to <i>S. noctilio</i>	<p>A. Brown crown with resin droplets on the bark due to oviposition. The crowns turn brown due to <i>A. areolatum</i> and phytotoxic mucus.</p> <p>B. Dead <i>S. noctilio</i> female abdomen found on the tree.</p> <p>C. Both indicate larval activity in the tree and indicated the current year's infestation,</p>
Missing trees	Planted seedlings and younger trees that never survived, or trees that were removed during a thinning operation,
Other	Trees with other symptoms that were not <i>S. noctilio</i> -related but are dead or dying due to some other biotic or abiotic factor (e.g. burnt, drought, lightning strike, natural mortality etc.),

6.2.2.4. Tree recording

Tree mortality due to *S. noctilio* was measured according to reduced point sampling in permanent sample plots, using the criteria in **Table 6.2**. Trees that died during the previous year or years were placed in one category as a historical record of *S. noctilio* damage. Trees that were dying due to *S. noctilio* activity in the current year, which did not have exit holes but showed all the symptoms of *S. noctilio* attack, were recorded under the current year's tree mortality. All living trees in the plot were assessed and the plots were demarcated with yellow paint to show the first and last tree in the line transect. The previous year's tree mortality and current year's mortality were expressed as a percentage of the living trees in the line transects. The NMI points were randomly selected within the pine-growing areas stretching from Cape Town to Louis Trichardt, with 90% in medium- to high-risk and 10% in low-risk plots.

Monitoring was carried out in the summer rainfall region of South Africa during March, April and May of each year, when host trees displayed signs of *S. noctilio* infestation. Inoculations of *D. siricidicola* took place from mid-February to May in the summer rainfall regions, with the NMI implemented once the inoculations were complete. Monitoring in the winter rainfall region was undertaken during May, June and July of each year. Signs of wasp oviposition varied between individual pine species. Pine species with higher resin content displayed resin streaks down the bark from the oviposition site, whereas those with lower resin content displayed resin beads at the oviposition site.

6.3. Results and Discussion

6.3.1. National monitoring during 2012 to 2014

Of the 535 plots surveyed by the reduced point method (**Fig. 6.4**), 482 were in *S. noctilio* high-risk areas (Croft, 2014). Some points were not assessed due to errors in data capture or because the areas proved unsuitable or inaccessible. Failed plots amounted to 171, 99 and 55 in 2012, 2013 (Germishuizen and Croft, 2014) and 2014, respectively.

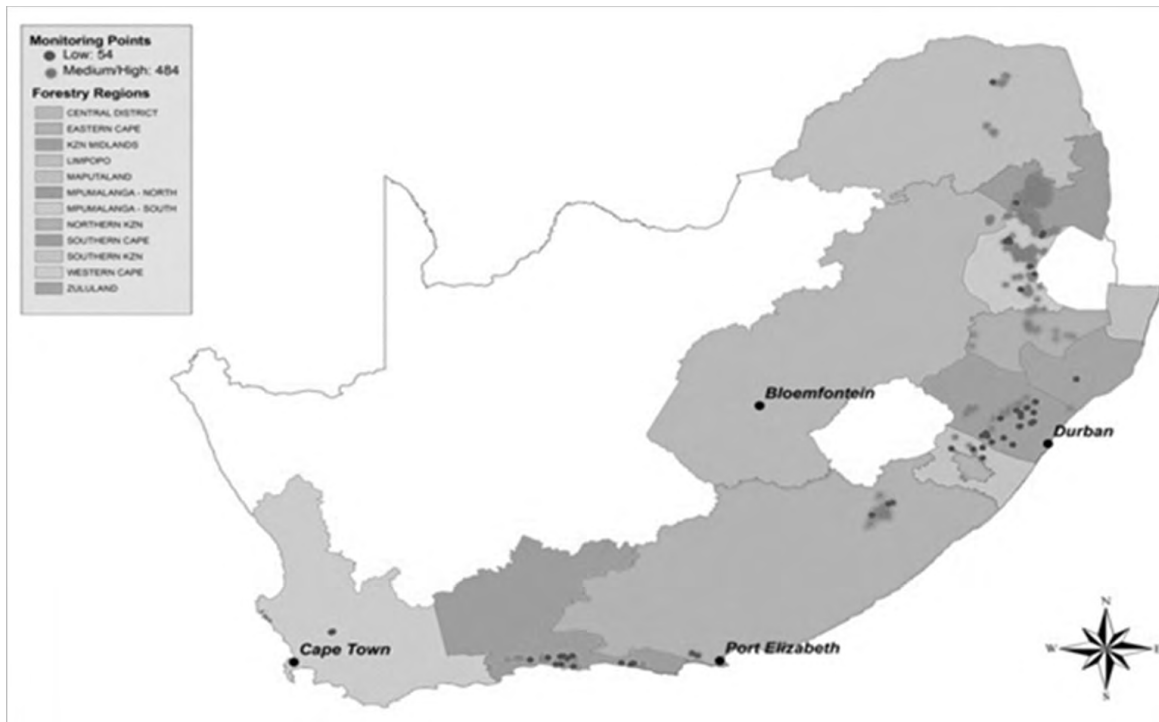


Figure 6.4. Distribution of 535 random points across South Africa where pine plantations were sampled for *S. noctilio* damage.

Of the 535 points sampled in 2012, 171 were not established (see above) resulting in the assessment of 364 sample points (**Table 6.3**). Of these, 73.4% showed no signs of dead or dying trees resulting from *S. noctilio* activity. Of the remainder, 15.7% had dead trees with no signs of dying trees, while 5.8% had signs of dying trees but no dead trees and 5.2% had both dying and dead trees (**Table 6.3**). The dying trees were mainly in the low ($\leq 5\%$), to moderate (5 - 10%) risk category (**Table 6.3**) (Croft, 2014).

In 2013, the database was updated to cater for changes in compartments resulting from fires, harvesting or plots that were inaccessible in 2012. As before, 99 points were not established and 436 points were assessed. The pattern was similar to 2012 in that there were no dead or dying trees in 72.5% of the sample points (**Table 6.3**). There were dying trees but no dead trees in 11.2%, dead trees and no dying trees in 5.2% and both dying and dead trees in 9.9% of the points. Dying trees were mainly in the medium-high risk (5 - 10%) category, with fewer in the low risk (1 – 5%) category (**Table 6.3**).

Table 6.3. Number of points with trees that were classified as dying, dead or both during the first three years of the NMI, according to the risk rating of the sites.

Year	Risk	No. of GIS Points	No. of sites	No. of Points with dead trees	No. of Points with no Dead trees	No. of Points with Dying trees	No. of Points with no Dying Trees	No. of points with Dead but no dying trees	No. of points with Dying but no dead trees	No. of Points with No Dead or Dying Trees	No. of points with Dead and Dying trees
2012		364	1759	76	288	40	324	57	21	267	19
	High	38	148	4	34	6	32		2	32	4
	Med High	44	143	7	37	14	30	3	10	27	4
	Low	282	1468	65	217	20	262	54	9	208	11
2013		436	1955	70	366	93	343	27	49	316	43
	Med High	392	1795	59	333	83	309	24	47	285	35
	Low	44	160	11	33	10	34	3	2	31	8
2014		480	2230	71	409	103	377	20	50	357	51
	Med High	367	1698	60	307	86	281	14	38	267	46
	Low	113	532	11	102	17	96	6	12	90	5
Total		1280	5944	217	1063	236	1044	104	120	940	113

In 2014, the database was updated and adjustments were made to cater for compartments that had been damaged or harvested. As before, some 55 points were inaccessible or unsuitable leaving 480 for assessment. Similar to previous years, there were no signs of dying or dead trees in 74.4% of the sample plots surveyed. There were dying trees and no dead trees in 10.4%, dead trees and no dying trees in 4.2% and both dying and dead trees in 10.6% of the points. Dying trees were mainly in the medium- to high-risk (5 – 10%) category when compared to the low-risk category (**Table 6.3**). Trends observed in the dying trees over the three years of monitoring can be seen in **Figure 6.5**.

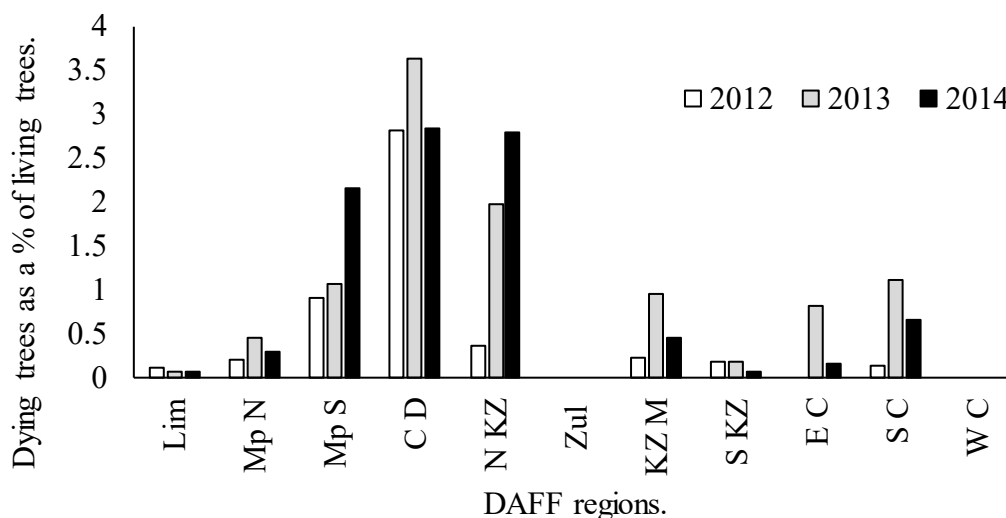


Figure 6.5. Dying trees expressed as a percentage of all living trees recorded in the National monitoring plots from 2012 to 2014, per DAFF region, where Lim = Limpopo, Mp N = Mpumalanga North, Mp S = Mpumalanga South, C D = Central District, N KZ = Northern KwaZulu-Natal, Zul = Zululand, KZ M = KwaZulu-Natal Midlands, S KZ = Southern KZN, E C = Eastern Cape, S C = Southern Cape and W C = Western Cape.

Increased *S. noctilio* activity from 2012 to 2014 as evidenced by an increase in the percentage of dying trees, was noted only in the Mpumalanga South and Northern KwaZulu-Natal regions (**Fig. 6.5**). In contrast, the Limpopo, Mpumalanga North, Central District, KZN Midlands, Eastern Cape and Southern Cape regions showed decreases in tree mortality from 2013 to 2014. This was presumably due to the success of the inoculations of *D. siricidicola* and the releases of *I. leucospoides* (**Chapter 3**).

Between 2012 and 2014, there was an improvement in the numbers of active (successful) monitoring points in the NMI (**Figure 6.6**). This trend was accomplished through improvements in the understanding and interpretation of the data from the different forestry companies, data recording in the field, communication with the contractors and awareness by the landowners.

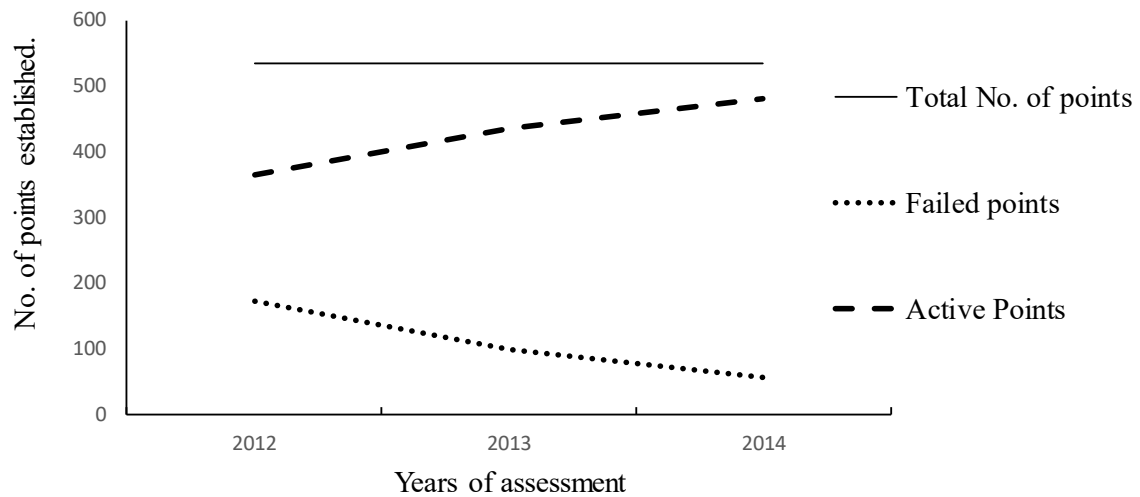


Figure 6.6. Establishment of active versus failed monitoring points during the NMI.

Each monitoring point has a GPS reference which is linked to other variables such as ownership, pine species, age, compartment number referring to a plantation map and hectares under plantation. However, for easy reference, each point was allocated a site number (**Table 6.6; Figure 6.7**).

Monitoring points that displayed tree loss (i.e. dead and dying) of more than 15% over the three years of monitoring were in the minority (**Figure 6.7**). The highest tree loss at any site was recorded in 2012 at 55%, but dropped to 32% and 35% in 2013 and 2014, respectively.

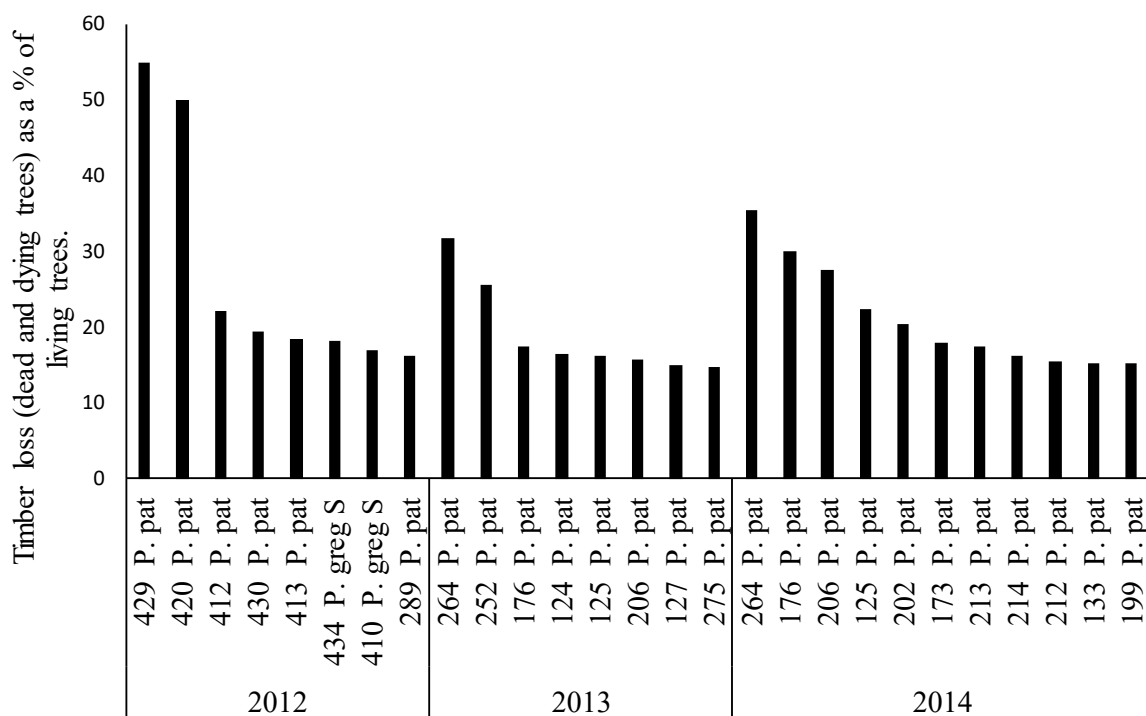


Figure 6.7. Individual site numbers where *Sirex noctilio*-related tree losses (dead and dying) were above 15%.

National monitoring points throughout South Africa that displayed exceptionally high percentages of dead or dying trees (i.e., above 10%) are indicated in **Table 6.6**. In 2013, the proportion of dead trees ranged from 0% to 20% while that of dying trees ranged from 0% to 19%. The highest percentages of dead trees were recorded in pulpwood plantations at three NMI points in northern KwaZulu-Natal (20%), Mpumalanga North (16%) and the KwaZulu-Natal Midlands (14%). The highest percentages of dying trees were recorded in pulpwood plantations at two NMI points in northern KwaZulu-Natal (12% and 19%) (**Table 6.6**).

By 2014, the NMI points with dying trees above 10 % (**Table 6.6**) were restricted to the Mpumalanga South region and measured 11-16%. Those with dead trees exceeding 10% (**Table 6.6**) were located in Mpumalanga North (14%), Mpumalanga South (12-20%), and Northern KZN (32%). These were all in pulpwood plantations except one point in Mpumalanga South (15%), which was a sawtimber plantation.

Table 6.6. National monitoring points throughout South Africa with exceptionally high percentages of dead or dying trees*.

Year of assessment	GIS		SPECIES	AGE	Risk	Latitude	Longitude	Sawtimber /		% Dead (Sirex)	% Dying (Sirex)
	Point number	Region						Pulpwood regime	No. of transects counted		
2013	252	Northern KZN	P. pat	16.0	Med High	-27.563102	30.488289	Pulpwood	3.00	80	19%
2013	264	Northern KZN	P. pat	18.2	Med High	-27.533311	30.548110	Pulpwood	3.00	83	12%
2013	124	Mpumalanga North	P. pat	16.4	Med High	-25.526412	30.439275	Pulpwood	3.00	83	16%
2013	133	Mpumalanga South	P. pat	14.1	Med High	-25.642510	30.613250	Pulpwood	3.00	80	11%
2013	176	Mpumalanga South	P. pat	15.3	Med High	-26.113402	30.408625	Pulpwood	3.00	84	11%
2013	264	Northern KZN	P. pat	18.2	Med High	-27.533311	30.548110	Pulpwood	3.00	83	20%
2013	275	KZN Midlands	P. pat	13.2	Med High	-28.981845	30.604139	Pulpwood	5.00	117	14%
2013	505	Southern Cape	P. rad	10.9	Med High	-33.945003	22.432914	Sawtimber	4.00	89	12%
2013	524	Southern Cape	P. rad	16.9	Med High	-34.017610	24.135620	Sawtimber	4.00	80	10%
2014	125	Mpumalanga South	P. pat	14.0	Med High	-25.727717	30.350956	Pulpwood	3.00	78	11%
2014	202	Mpumalanga South	P. pat	15.9	Med High	-26.389342	30.699958	Pulpwood	3.00	102	11%
2014	206	Mpumalanga South	P. pat	14.1	Low	-26.442234	30.673393	Pulpwood	3.00	112	13%
2014	209	Mpumalanga South	P. pat	12.8	Med High	-26.449236	30.679284	Pulpwood	3.00	111	11%
2014	212	Mpumalanga South	P. pat	13.7	Med High	-26.485580	30.646373	Pulpwood	2.00	83	11%
2014	213	Mpumalanga South	P. pat	16.1	Med High	-26.496459	30.586442	Pulpwood	3.00	107	16%
2014	124	Mpumalanga North	P. pat	17.4	Med High	-25.526412	30.439275	Pulpwood	3.00	86	14%
2014	125	Mpumalanga South	P. pat	14.0	Med High	-25.727717	30.350956	Pulpwood	3.00	78	12%
2014	173	Mpumalanga South	P. pat	15.1	Med High	-26.048372	30.367459	Pulpwood	3.00	100	13%
2014	176	Mpumalanga South	P. pat	16.3	Med High	-26.113402	30.408625	Pulpwood	3.00	84	20%
2014	199	Mpumalanga South	P. pat	21.1	Med High	-26.354694	30.584888	Sawtimber	8.00	87	15%
2014	206	Mpumalanga South	P. pat	14.1	Med High	-26.442234	30.673393	Pulpwood	3.00	112	14%
2014	264	Northern KZN	P. pat	19.0	Med High	-27.533311	30.548110	Pulpwood	3.00	83	32%

* Where the average age of the *Pinus patula* (P. pat) and *Pinus radiata* (P. rad) trees are indicated in years.

Points (27 in total) where dead and dying trees exceeded 15% (**Figure 6.7**) were largely located in highly stocked pulpwood rotations except for one sawtimber point, namely 199 (**Table 6.6**), where five and eight transects were laid out to ensure that sufficient trees were assessed. Both NMI points in sawtimber compartments were at the lower percentage range (10-15% dead trees), which again showed that highly stocked pulpwood regimes (11-32%) were more susceptible to *S. noctilio* infestation.

There was also one pulpwood point (NMI point 212 in 2014) where two transects were assessed, due to very high stocking (**Table 6.6**). Finding *S. noctilio* at this site was not surprising. The few highly infested sawtimber points in relation to pulpwood points (**Table 6.6**) supports the contention that pulpwood trees are more attractive to *S. noctilio*. This is most likely due to inter-tree competition for water, nutrients and light which places the smaller trees under nutrient and water stress (Madden, 1988).

The dominant species at these 27 highly-impacted points was *P. patula*, with only two NMI points involving a different species, namely the southern variation of *P. greggii* (**Figure 6.7**). This indicates that *P. greggii* is susceptible to *S. noctilio* albeit less than *P. patula*.

In **Table 6.6**, point 206 in 2014 was categorised as having a low climatic risk, while all the other plots were at medium to high risk indicating that silvicultural practices need to be considered when assessing sites.

Although dead trees were removed in subsequent thinning operations by sawtimber producers, the percentage of NMI points that displayed no tree mortality increased over the three year period (**Figure 6.8**). The points with dead trees decreased from 21% in 2012 to 15% in 2014 and those with dying trees increased from 11% in 2012 to 21% in 2014. The increase in the incidence of dying trees was more realistic in that the sawtimber companies had removed the dead trees in thinnings before the assessments were carried out. This increase indicated that by 2014 insufficient parasitism by *D. siricidicola* and *I. leucospoides* had taken place.

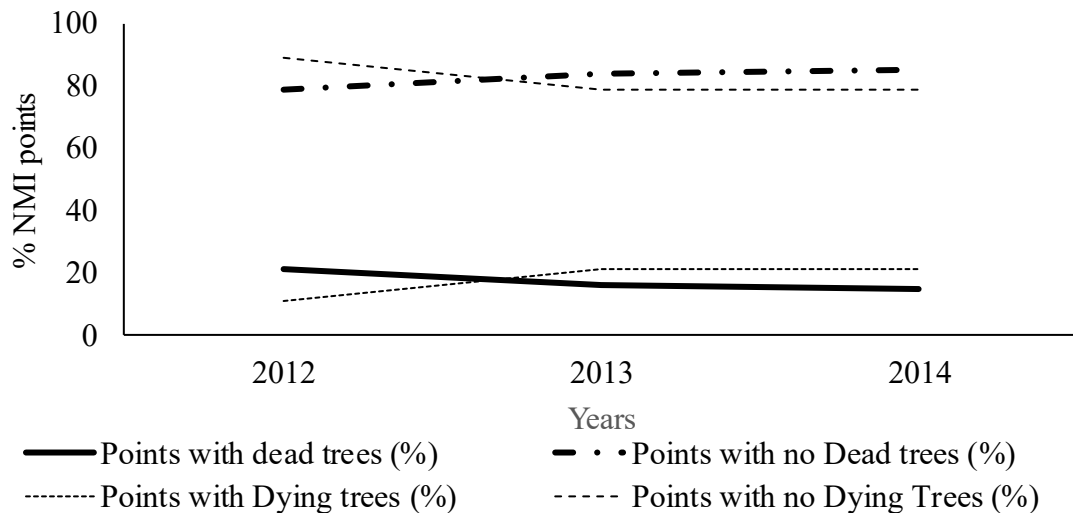


Figure 6.8. Percentage of NMI points that recorded dead and dying trees due to *S. noctilio* infestation during 2012 to 2014.

There was a similar pattern in the percentage of NMI points that displayed no dying trees over the three years (**Figure 6. 8**). Since eradication of *S. noctilio* is not possible, the number of dying trees should never be allowed to decrease below 1% so that the populations of *D. siricidicola* and *I. leucospoides* cannot be supported.

6.4. Conclusions

The NMI provided an annual assessment of *S. noctilio*-related damage per region, thereby highlighting regions where intervention was required, by comparing the results between 2012 and 2013, and between 2013 and 2014. Overall, *S. noctilio*-related dead and dying tree damage was below 5%, during all three years in all regions, indicating that the *S. noctilio* populations in South Africa were below the threshold that defines the onset of an outbreak (Villacide and Corley, 2012). The relatively low damage levels also confirmed that the integrated management strategy adopted by the SASCP had been successful. However, the Northern KwaZulu-Natal and Southern Mpumalanga regions had areas with higher levels of *S. noctilio* activity in 2012 and 2013, where the percentage of dying trees at the NMI points ranged from 5% to 10%. Of interest was the Eastern Cape region, where tree mortality reached as high as 55% in 2012. The future focus of the SASCP should be to change the

trend of high infestation levels in the Mpumalanga South and Northern KwaZulu-Natal regions and ensure that the other regions maintain the current trend of low infestation levels.

The NMI proved to be a useful and necessary part of the national integrated management strategy for *S. noctilio*. It also provided an excellent tool for quantifying the *S. noctilio*-related tree damage as an indication of the *S. noctilio* population and highlighting regions where intervention through biological control was necessary. The benefits of the NMI are that SASCP had a tree mortality measurement, by region, across South Africa and could assess tree mortality annually according to the previous year's results. These NMI results assisted in determining the quantities of *D. siricidicola* and *I. leucospoides* that were required to decrease *S. noctilio* populations.

Monitoring has many facets, and the NMI, together with the emergence cage and panel trap monitoring, provided an overall assessment of the spread and infestation levels of *S. noctilio* in South Africa. These ensured a comprehensive understanding of the size of the *S. noctilio* populations at national and regional levels. The size of the populations were expressed in terms of dying and dead trees for each climate risk group across the country.

7.0. THE STATE OF *SIREX NOCTILIO* IN SOUTH AFRICA IN 2014

7.1. Introduction

Since its formation in 2004, the various components of the SASCP, as presented in the preceding chapters, were all carried out to provide sufficient information to the South African Forestry Industry on the spread (spatially and in terms of population density) of *S. noctilio* and the effects thereof. The SASCP has been successful in the collection of data that have supported the management of this pest in South Africa through biological control measures.

7.2. Overview of the components of the SASCP

7.2.1. Panel trap network in South Africa

The monitoring of *S. noctilio* in South Africa started with the use of panel traps, and played a vital role in understanding the spread of *S. noctilio* from 2007 to 2011. However, since panel traps only indicated the presence or absence of *S. noctilio*, an estimation of wasp population size was not possible since the area of attraction by the lure was not measurable and factors such as wind direction, wind speed, and localised swarming of *S. noctilio* all affected the efficiency of the traps (Croft *et al.*, 2014). It was evident from the trap network data that individual traps that were only 100 m apart (three traps per site), caught different numbers of *S. noctilio*. Other indicators were thus used to obtain a quantitative measure of the wasp's population sizes. For example, the number of dying and/or dead trees provided information on the impact of the local *S. noctilio* population while the number of wasps per sampled log gave an indication of the population size.

7.2.2. Quantitative tree damage assessments (NMI)

The NMI provided a quantitative assessment of annual tree damage and determined the number of dead and dying trees per annum at selected sample sites. Since dead trees indicate tree mortality in previous years, the numbers of dead trees at the sample sites were assessed over time to determine increased or decreased rates of tree mortality. The presence of dying

trees indicated *S. noctilio* damage in the current year of assessment (Croft *et al.*, 2014). Such assessments of dying trees were representative of financial loss, especially if the NMI assessments recorded tree diameter at breast height, from which a volume loss per annum could be calculated and reported.

7.2.3. Emergence cage assessments

The data collected from the emergence cages provided vital information on the annual levels of parasitism by *D. siricidicola*. This was very important to gauge the degree of success achieved in reducing the *S. noctilio* population and the assurance of sustained population suppression if parasitism remained sufficiently high (above 20%) (Croft *et al.*, 2014).

Furthermore, information on the number of wasps per log indicated the size of the population at the sample area, where 4-5 wasps per log was considered a low population density, while numbers reaching 25 wasps per log was considered high. Emergence of *I. leucospoides* from the logs also confirmed the establishment of the parasitoid at the sampled location.

All aspects of SASCP monitoring in South Africa were recorded with a company, farm and compartment label, which facilitated specific site management decisions. Emergence cage results that revealed the absence of *I. leucospoides* and nematode-infected *S. noctilio* indicated that biological control was not active within the area of assessment. Such areas were subsequently prioritized for the deployment of biocontrol agents.

7.2.4 Overall trends revealed by monitoring

Since different monitoring methods were deployed, which individually could have revealed different and misleading trends, the data were combined to provide an overall assessment. Between 2012 and 2014, data were compiled from the emergence cage results (numbers of parasitized and unparasitized *S. noctilio* and numbers of *I. leucospoides*) and the NMI results.

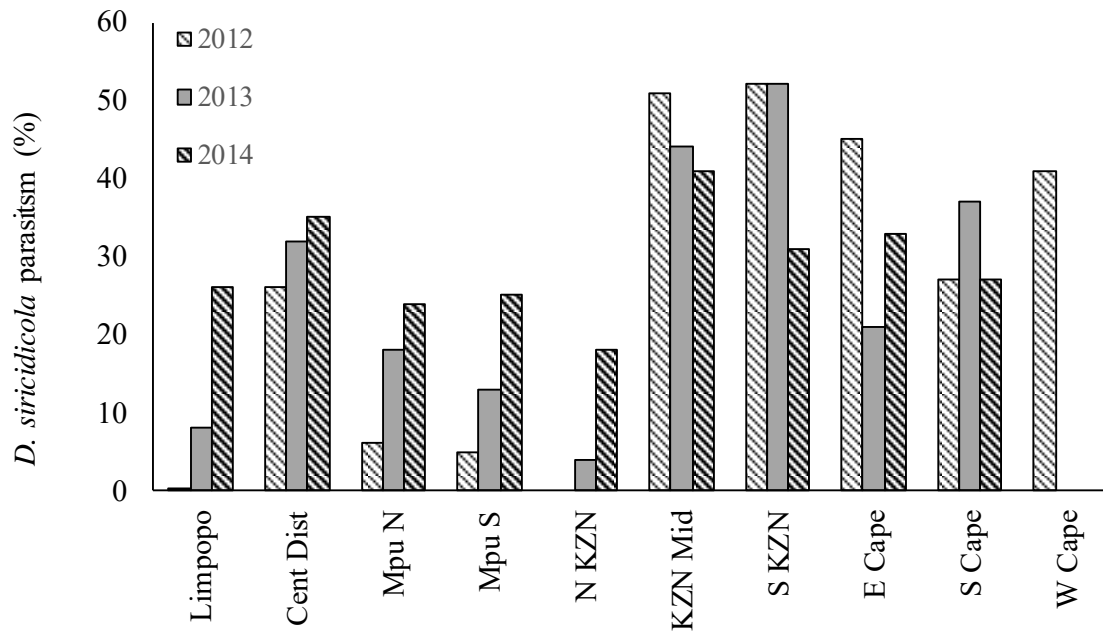


Figure 7.3. Percentage of *S. noctilio* wasps that were parasitized by *D. siricidicola* in the different DAFF regions from 2012 to 2014.

Parasitism by *D. siricidicola* increased in most of the DAFF regions during 2012-2014 (Figure 7.3). However, these trends were not apparent in three DAFF regions, namely the Southern Cape, Southern KwaZulu-Natal and KwaZulu-Natal Midlands. Despite decreased parasitism in these three regions, overall levels were above 20% in all three and above 30% in two. Although 2014 parasitism levels were above the critical 10% level in all DAFF regions (Figure 7.3), the three regions that displayed reduced parasitism need further monitoring and possibly further inoculation of *D. siricidicola* to ensure that critical parasitism levels are sustained.

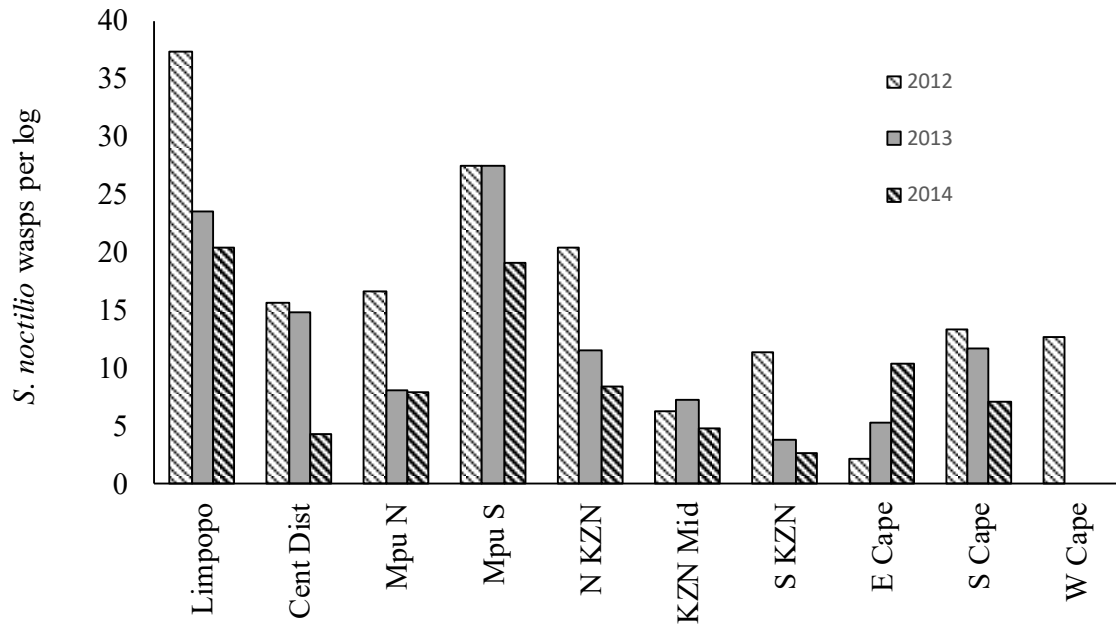


Figure 7.4. Numbers of *Sirex noctilio* wasps per log in the different DAFF regions from 2012 to 2014.

Wasp emergence declined in all but one of the DAFF regions during 2012-2014 (**Figure 7.4**) indicating the efficacy of the nematode-inoculation efforts. Wasp emergence increased over time in the Eastern Cape region. However, this region displayed the second lowest numbers of wasps per log overall, but should still be monitored to ensure that this increasing trend does not continue. The industry is currently (2016) accepting a norm of two females per 0.8 m log as acceptable to continue the spread of *D. siricidicola* while higher numbers could indicate a population increase (Verleur *et al.*, 2016). Since the average sex ratio was 5.7 males per female in South Africa (**Table 3.4**), an acceptable number of wasps per log would be below the average of 11.4 wasps.

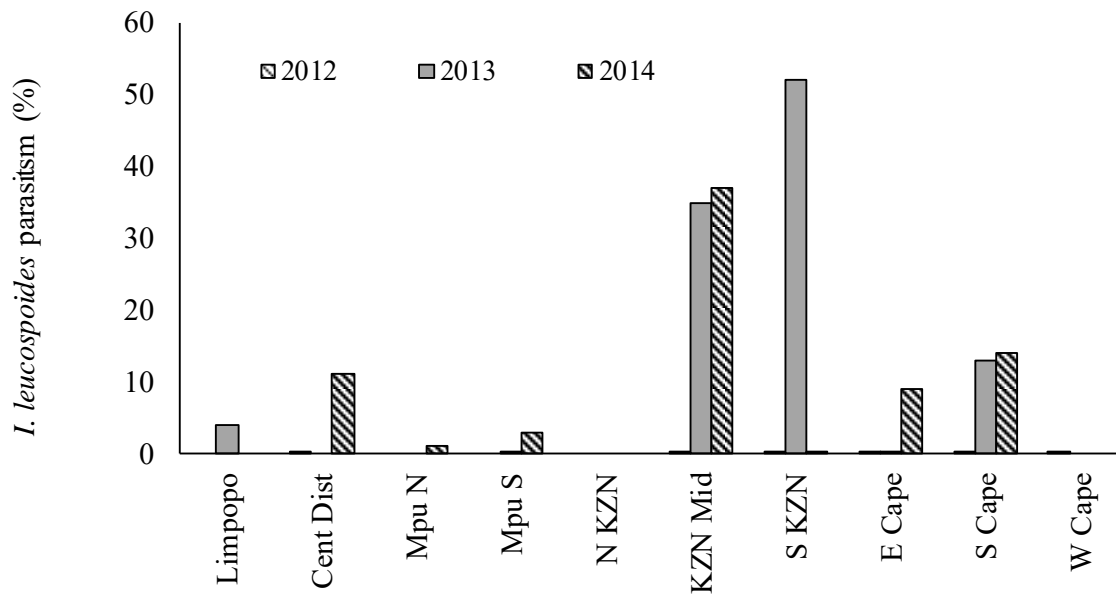


Figure 7.5. Percentage of *Sirex noctilio* that were parasitized by *Ibalia leucospoides* in the different DAFF regions from 2012 to 2014.

The larval parasitoid *I. leucospoides* was recovered from all but one of the DAFF regions (Figure 7.5). Percentage parasitism by 2014 was below 10% in four DAFF regions. The highest levels of parasitism were recorded in the KZN Midlands, Southern KZN and Western Cape regions. The Western Cape region has never displayed high tree mortality from *S. noctilio*, and logs were difficult to find in this region; therefore, results for the Western Cape are often missing from figures. Increasing trends of parasitism were recorded in most regions, except for the Northern KZN and Western Cape where levels were similar and very low between years and the Western Cape and Limpopo which displayed decreased levels (Figure 7.5). The Southern Cape region previously supported large populations of the parasitoid and the current trend may be indicative of the NMI data where the numbers of dying trees was reduced (Figure. 7.6). Limpopo was the last region where *I. leucospoides* was released and the population probably requires more time to increase.

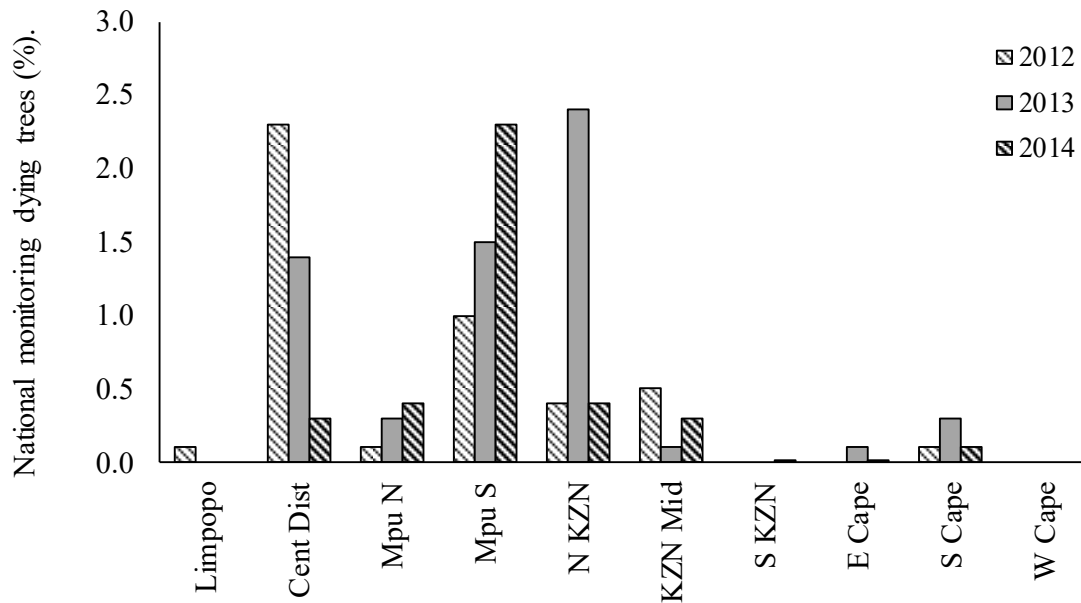


Figure 7.6. Percentages of pine trees dying from *Sirex noctilio* infestation in the different DAFF regions from 2012 to 2014, as determined by the NMI.

The NMI revealed trends of decreasing percentages of dying trees in most of the DAFF regions, where the percentages of dying trees were <1% by 2014 (**Figure 7.6**). However, increases in dying trees were recorded in Mpumalanga South and to a lesser extent in Mpumalanga North. The percentage of dying trees in a region is considered as acceptable when below 1%. This increase was more severe in Mpumalanga South where up to 2.3% of the trees were dying, compared with <1% of trees in Mpumalanga North (Germishuizen and Croft, 2014). Additional inoculations were carried out in Mpumalanga South in 2015, but the outcomes of these have not been included in this assessment.

While the above regional assessment was insightful, a national perspective was required to prioritize the regions that were most at risk to *S. noctilio* losses.

7.3. National perspective on *Sirex noctilio*

To obtain a national perspective on *S. noctilio* in South Africa, the four monitoring parameters were evaluated together with the *S. noctilio* climatic risk rating (Germishuizen *et*

al., 2013) for each region in a consolidated table (**Table 7.1**) that prioritised the DAFF regions according to the severity of the risk. The final prioritisation per DAFF region was somewhat subjective, as the weighting for each parameter need not be equal if a cost-benefit approach is considered. Nonetheless, it is reasonable to expect that the population densities of *I. leucospoides* and *D. siricidicola* should be weighted equally. The number of wasps per log (from emergence cage data) provided a good indication of the *S. noctilio* population size and this matched, to some degree, the NMI results that indicated the numbers of dying trees. The ideal situation in each region is high rates of parasitism by *D. siricidicola* and *I. leucospoides*, together with low numbers of wasps per log and a low percentage of dying trees determined by the NMI. The Southern KwaZulu-Natal region was probably the closest to the desired norm, followed by the Eastern Cape and KwaZulu-Natal Midlands regions.

The final assessment for 2014 ranked the Mpumalanga South region as having the highest priority (**Table 7.1**) for additional biological control efforts to reduce the number of wasps per log and hence the percentage of dying trees. The second highest priorities were the Mpumalanga North and Northern KwaZulu-Natal regions since, despite high levels of parasitism from *D. siricidicola*, parasitism from *I. leucospoides* was low to zero with some dying trees and high numbers of wasps per log being the main concern.

The third highest priorities were the KwaZulu-Natal Midlands, Central Districts and Limpopo regions (**Table 7.1**) where there were reduced levels of parasitism by *D. siricidicola*, although all three regions were at an acceptable level in terms of *S. noctilio* numbers and dying trees. The number of wasps per log was exceptionally high in Limpopo making it an area of concern that required further releases of *D. siricidicola* and *I. leucospoides*; particularly since there was no evidence of *I. leucospoides* establishment. The concern in these regions is the percentage of dying trees per hectare.

Five DAFF regions were ranked as having the lowest priority and these include Southern KwaZulu-Natal, Eastern Cape, Southern Cape, Western Cape and Zululand (Table 7.1). This was largely based on high rates of parasitism by *D. siricidicola* and *I. leucospoides*.

Although there were variable numbers of wasps per log in these regions, the levels of parasitism reduce the risks of any *S. noctilio* outbreaks. The percentage of dying trees in these regions was low and acceptable. Although control of *S. noctilio* has been achieved in several regions, additional efforts are required to ensure that this is consistent and sustained across South Africa.

Table 7.1. Prioritization of DAFF regions in South Africa in relation to their susceptibility to *Sirex noctilio*, based on consolidated data from the SASCP from 2012 to 2014*.

Region in South Africa	<i>D. siricidicola</i> parasitism			<i>S. noctilio</i> wasps per log			<i>I. leucospoides</i> parasitism			National monitoring Dying trees			Risk rating		Priority
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014	Low	High	
Mpu S	5.00	13.00	25.00	27.5	27.4	19.0	0.00	0.00	3.00	1.00	1.50	2.30	1 Low	3 High	1
Mpu N	6.00	18.00	24.00	16.6	8.0	7.9	0.00	0.00	1.00	0.10	0.30	0.40	1 Low	3 High	2
N KZN	0.00	4.00	18.00	20.4	11.5	8.3	0.00	0.00	0.00	0.40	2.40	0.40	1 Low	2 Moderate	2
KZN Mid	51.00	44.00	41.00	6.3	7.1	4.7	37.00	35.00	37.00	0.50	0.10	0.30	1 Low	4 Very High	3
Cent Dist	26.00	32.00	35.00	15.5	14.9	4.3	0.00	0.00	11.00	2.30	1.40	0.30	1 Low	2 Moderate	3
Limpopo	0.00	8.00	26.00	37.3	23.5	20.4	0.00	4.00	0.00	0.10	0.00	0.00	1 Low	2 Moderate	3
S KZN	52.00	52.00	31.00	11.3	3.8	2.7	19.00	52.00	0.39	0.00	0.00	0.00	1 Low	4 Very High	4
E Cape	45.00	21.00	33.00	2.1	5.3	10.4	17.00	0.00	9.00	0.00	0.10	0.00	1 Low	2 Moderate	4
S Cape	27.00	37.00	27.00	13.4	11.7	7.1	14.00	13.00	14.00	0.10	0.30	0.10	1 Low	3 High	4
W Cape	41.00			12.6			36.00			0.00	0.00	0.00	1 Low	3 High	4
Zululand										0.00	0.00	0.00			5
							Positive	Negative			Neutral				

* The highlighted areas indicate the initial monitoring results and the subsequent monitoring results where there was no change. The numbers in bold print indicate positive outcomes while those in normal print indicate negative outcomes when compared to the previous year.

7.4. Conclusions

These combined results indicate that most of the pine-growing regions of South Africa have experienced some level of biological control from *D. siricidicola* and *I. leucospoides*.

However, other regions such as Mpumalanga South, Mpumalanga North and Northern KwaZulu-Natal still require careful monitoring and further increases in parasitism levels before the objectives of the SASCP are fully met.

In South Africa, the short-rotation pine pulpwood plantations will always need monitoring to ensure that tree mortalities are minimized and that new outbreaks of *S. noctilio* are prevented.

This can be achieved by releasing *D. siricidicola* and *I. leucospoides* as soon as the percentages of dying trees and parasitism levels indicate that these interventions are required.

The quantities of *D. siricidicola* and *I. leucospoides* that are released annually will probably decrease in the immediate future because of the parasitism levels already achieved.

However, monitoring must continue if South Africa maintains pine pulpwood and sawtimber plantations where the presence of *S. noctilio* and its related damage remains evident.

Research into more effective biological control measures should continue to ensure higher levels of parasitism than those provided by the present Kamona strain of *D. siricidicola*.

Other countries have reported higher levels of parasitism following the first releases of this strain of *D. siricidicola*, leading to suspicions that there are climatic barriers to higher parasitism in South Africa (Hurley *et al.*, 2007). There are also possibilities for the release of additional parasitoids that have been released against *S. noctilio* elsewhere in the world, but these still need to be fully investigated and tested.

The full impact of future climate change may result in shifts in the risk ranking of the various DAFF regions, since the *S. noctilio* risk model is based on climatic factors. This highlights the importance of continued monitoring, such as implemented by the SASCP, to ensure that future outbreaks of *S. noctilio* are curtailed.

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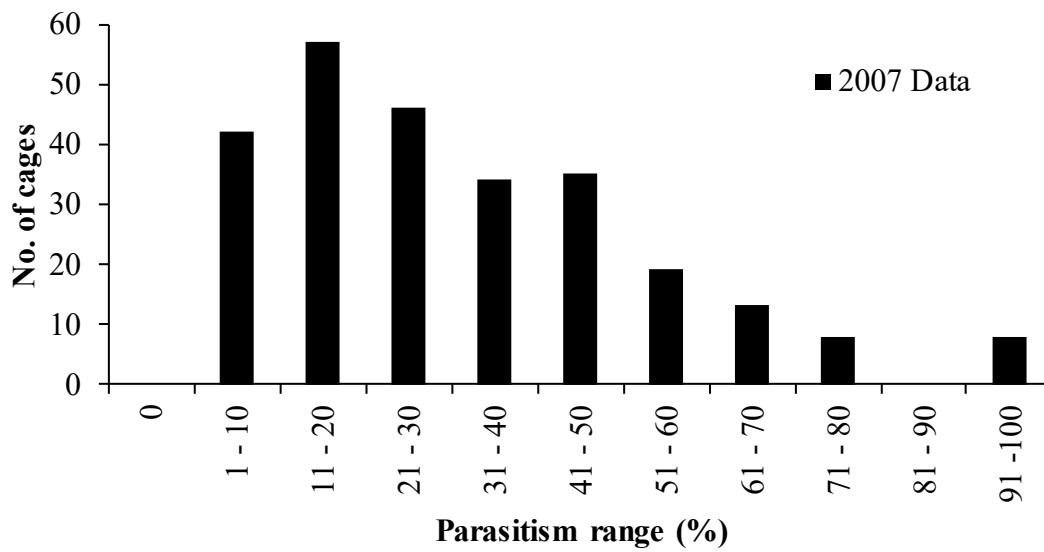
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9.0. APPENDICES

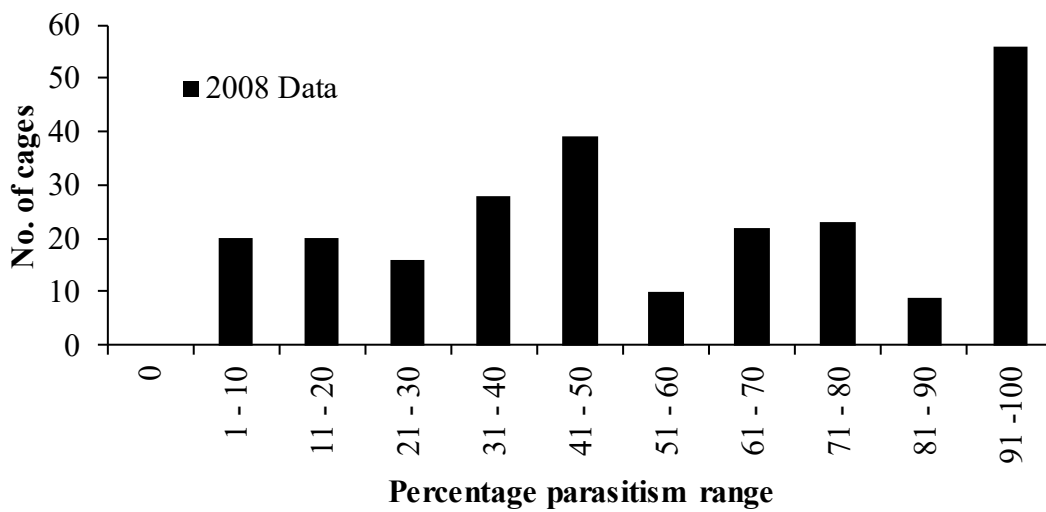
9.1. Appendix 1

Parasitism trends over time since the inception of the depot are illustrated by the graphs below for each of the depots, per year from the time the depot was created up to 2014.

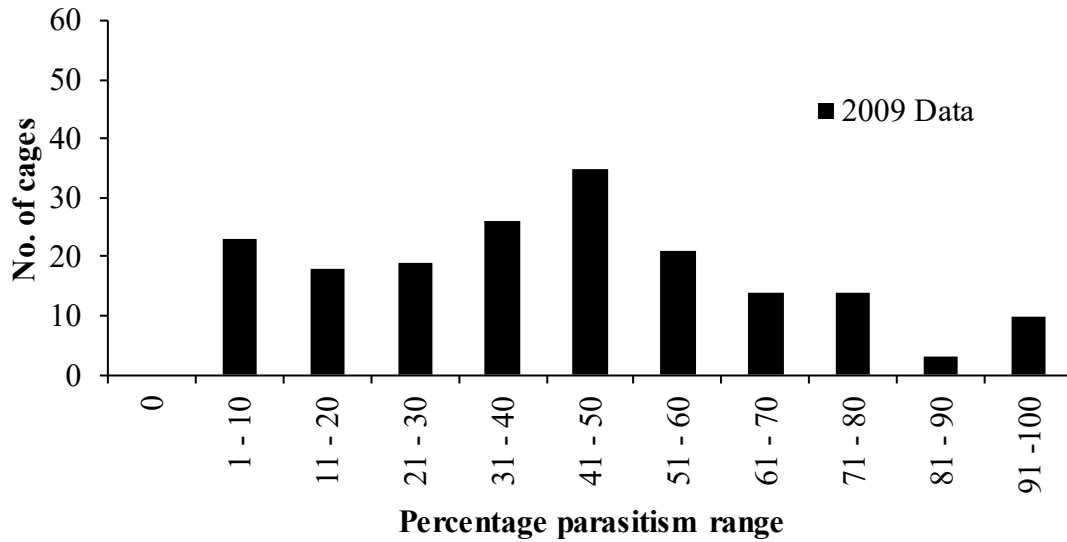
9.1.1. Linwood Depot percentage frequency graph for 2007.



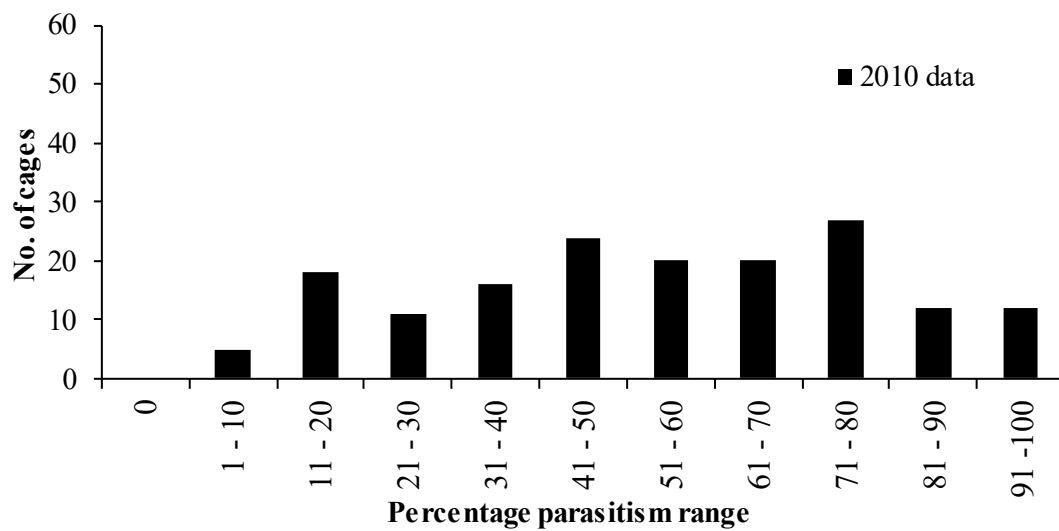
The initial frequency is at the lower percentage ranges



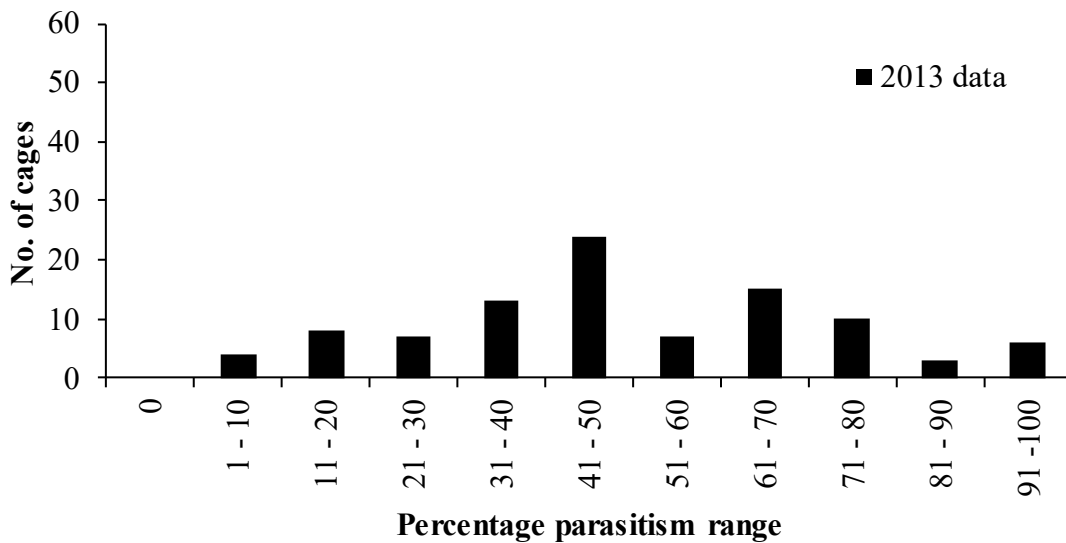
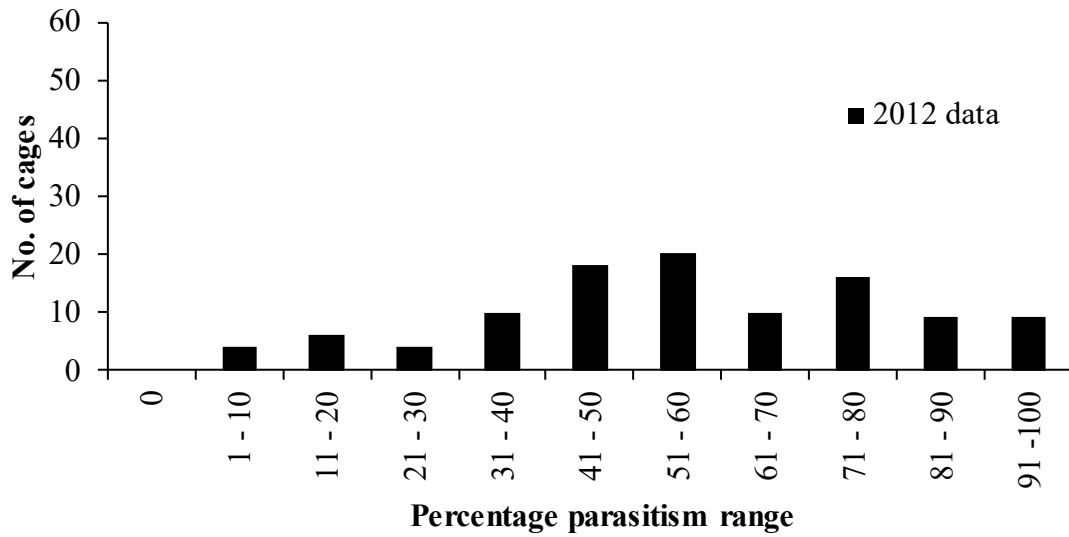
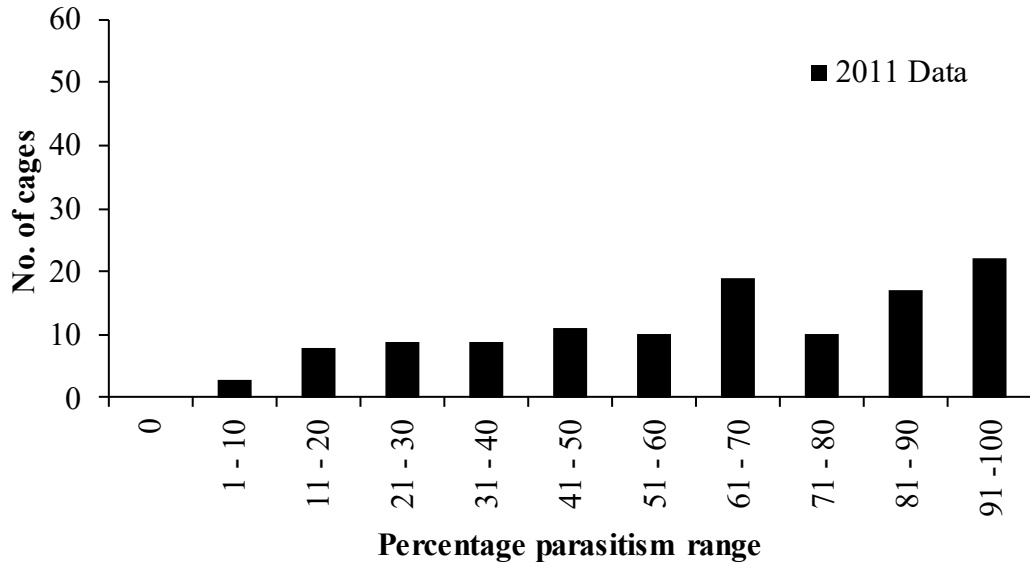
One year later the majority are in the 91 % to 100 % category, which was a positive development.

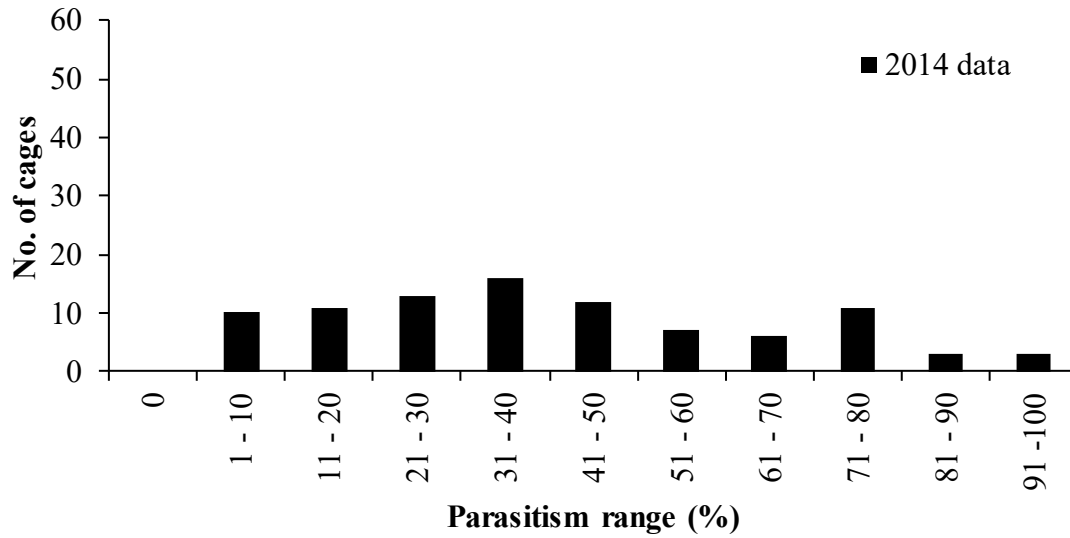


The 2009 data represents a more normal situation with the parasitism frequency around the 50 % level.

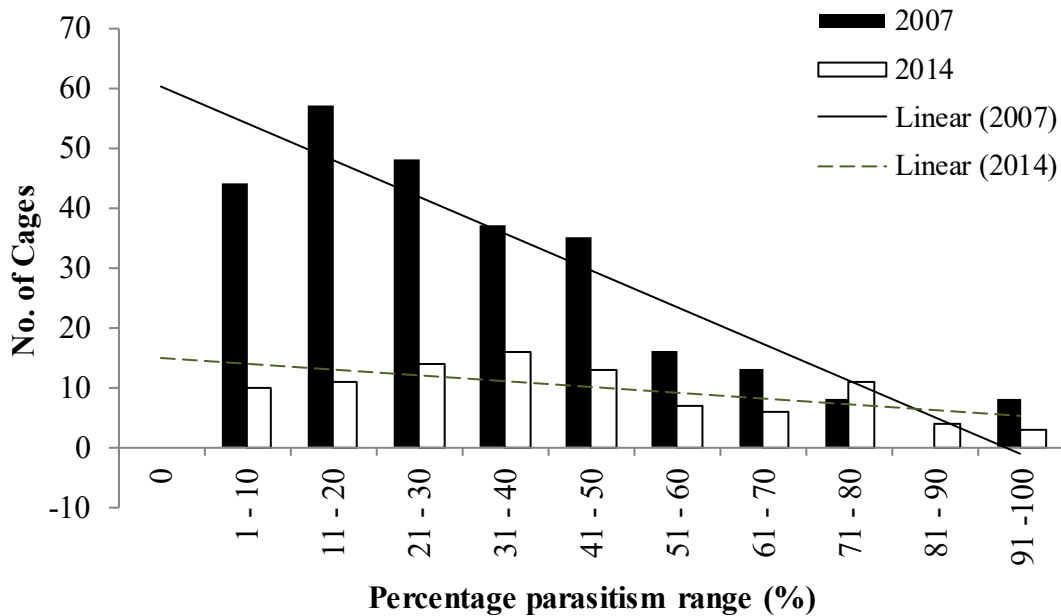


Here there was another move to the higher percentage ranges.

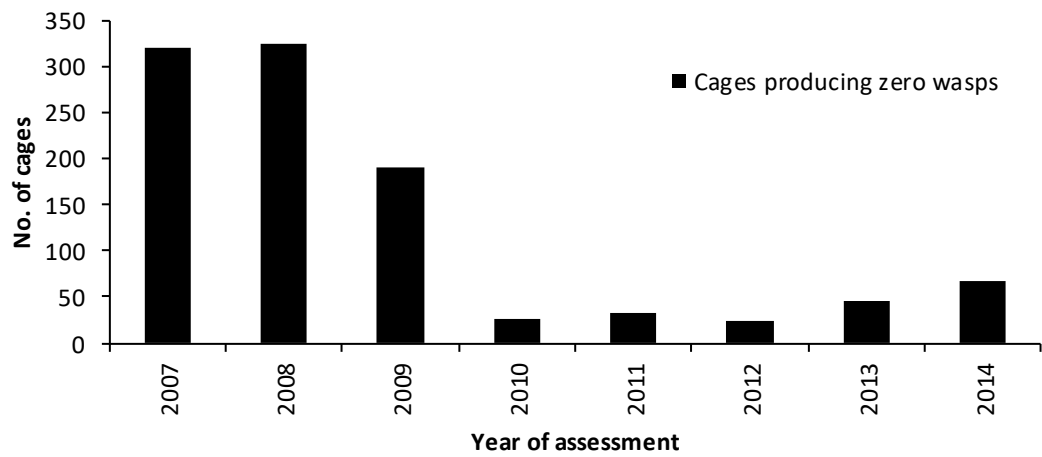




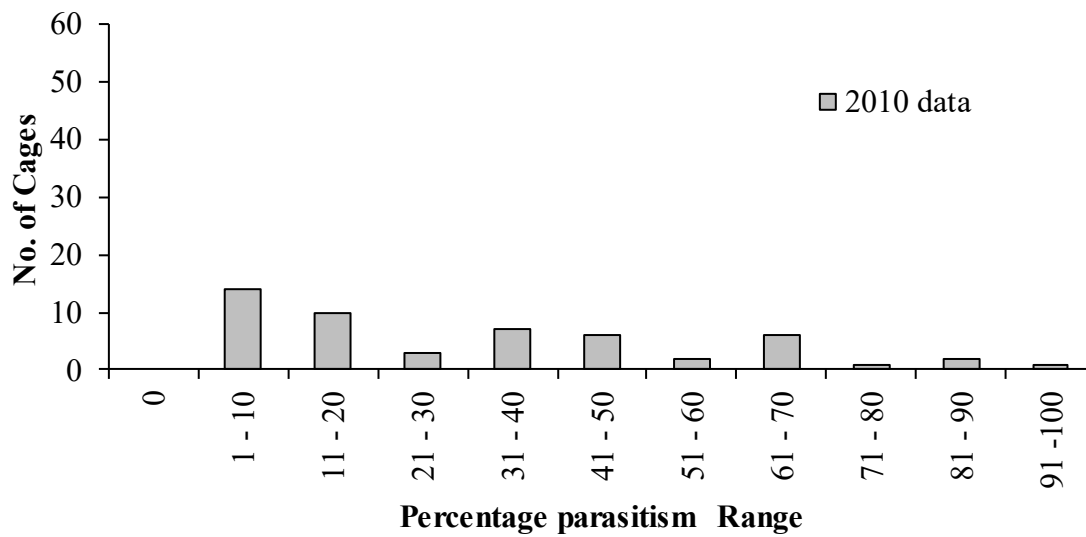
The change in parasitism frequency at Linwood between 2007 and 2014 moved in a beneficial manner with fewer cages having lower parasitism percentages and more cages measuring higher parasitism. The graph below indicates the trend lines between the two years.

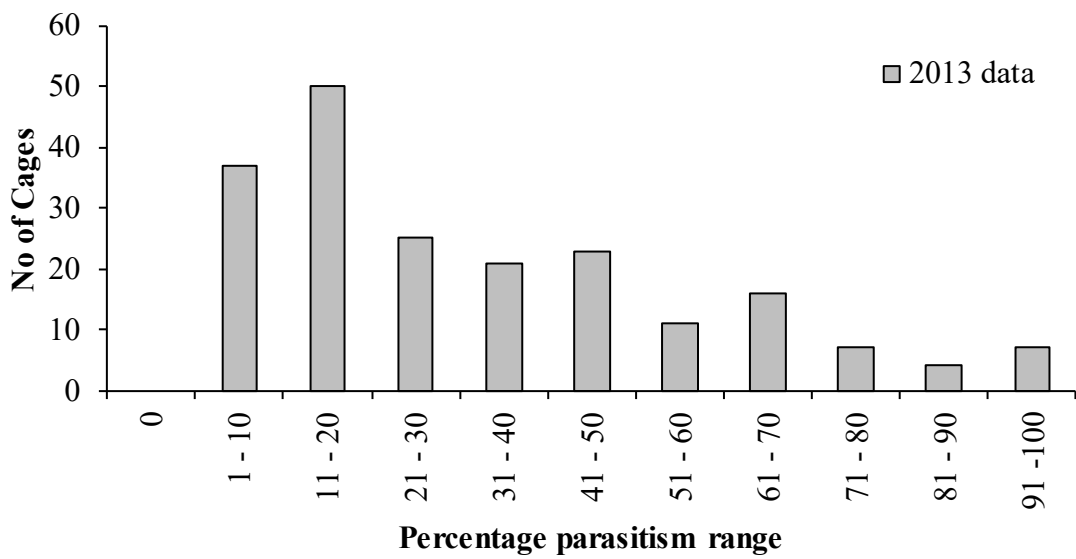
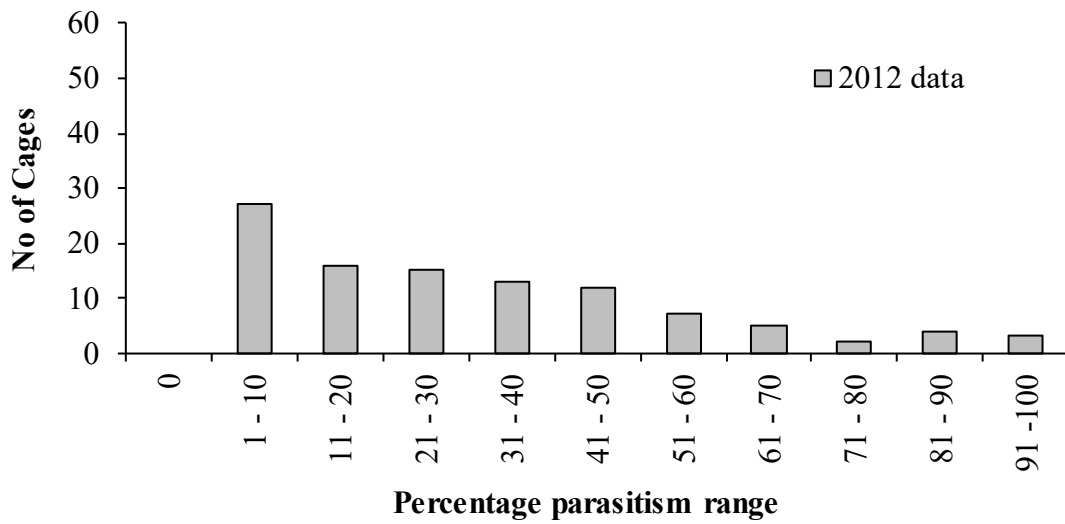
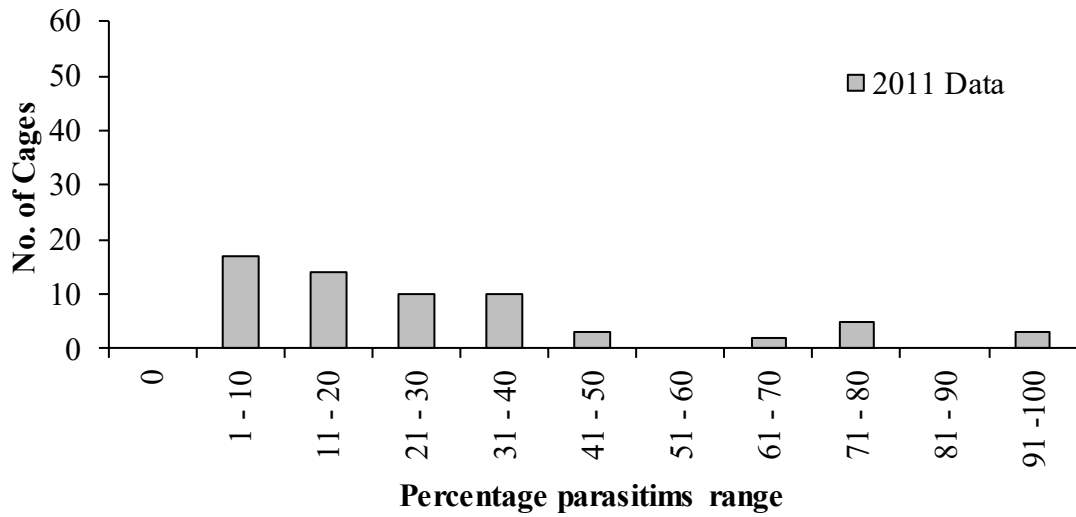


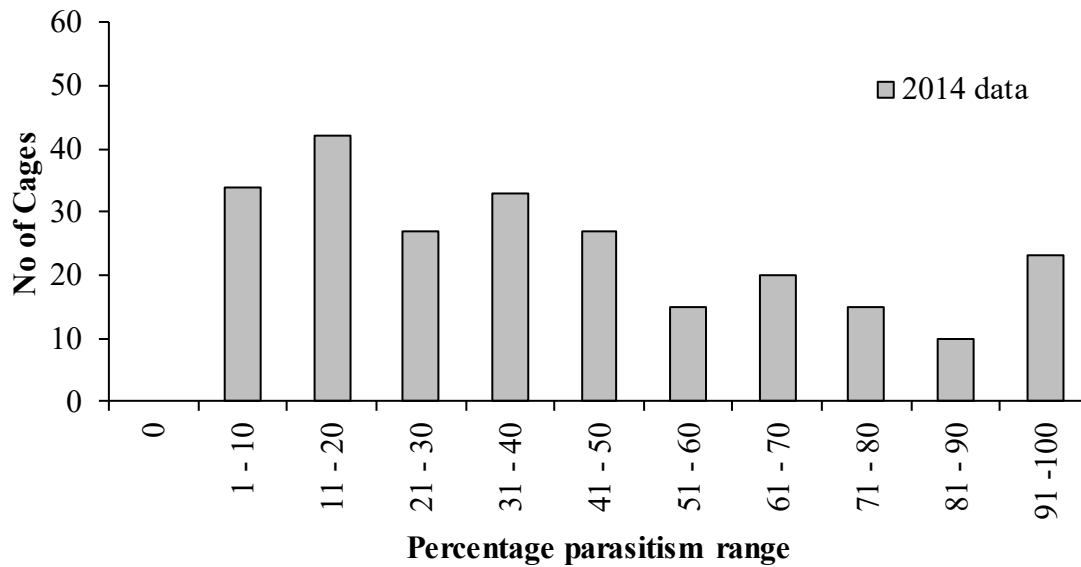
The 2007 trend line was heavily leaning to the lower percentage ranges. Fortunately, by 2014 the trend has become more normalised and the line indicates a healthy range of parasitism throughout the percentage ranges.



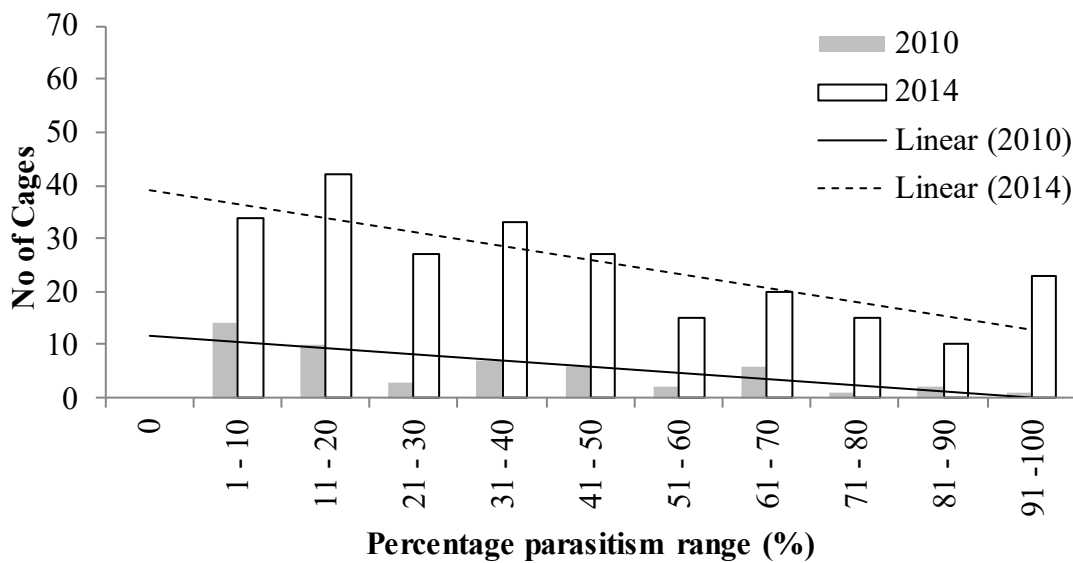
9.1.2. Vryheid depot data

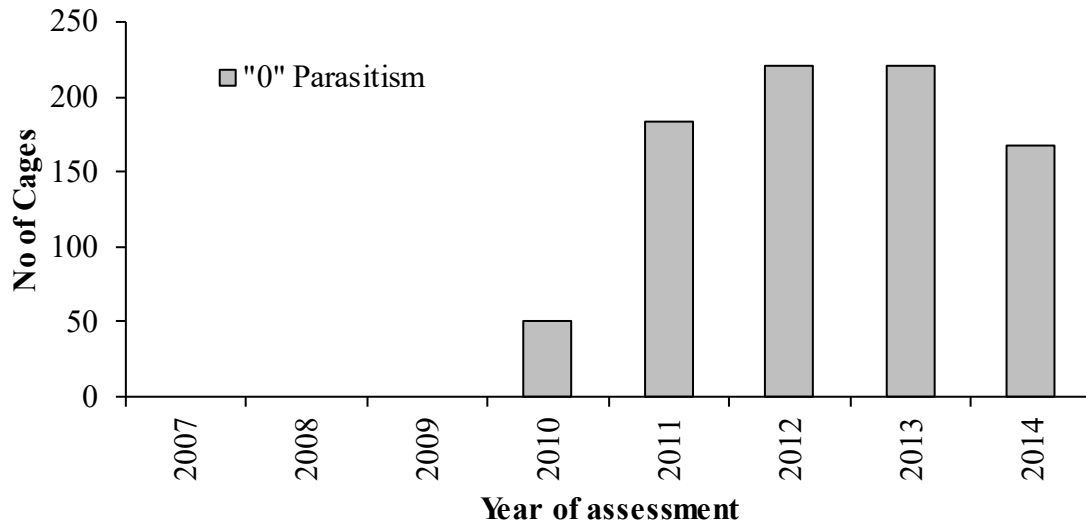




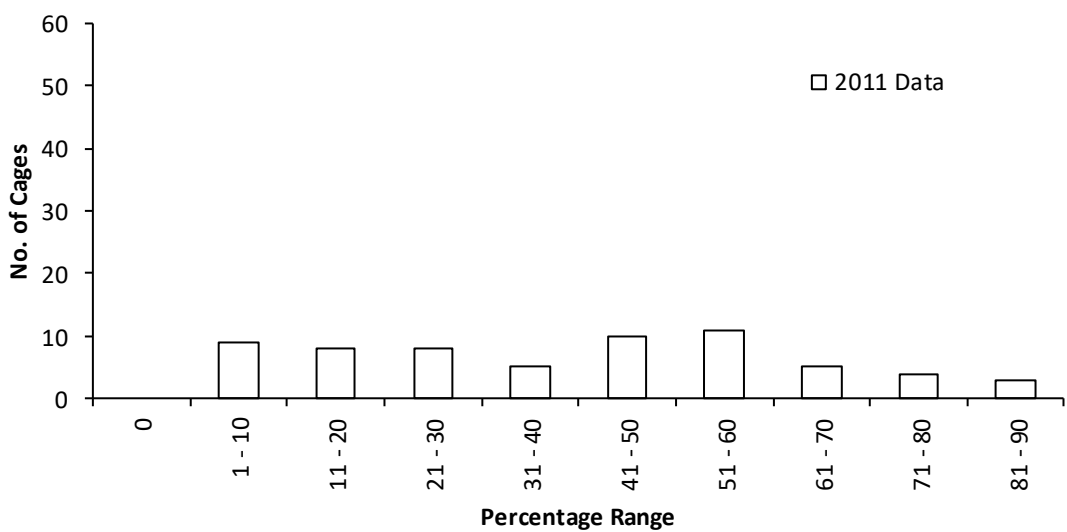
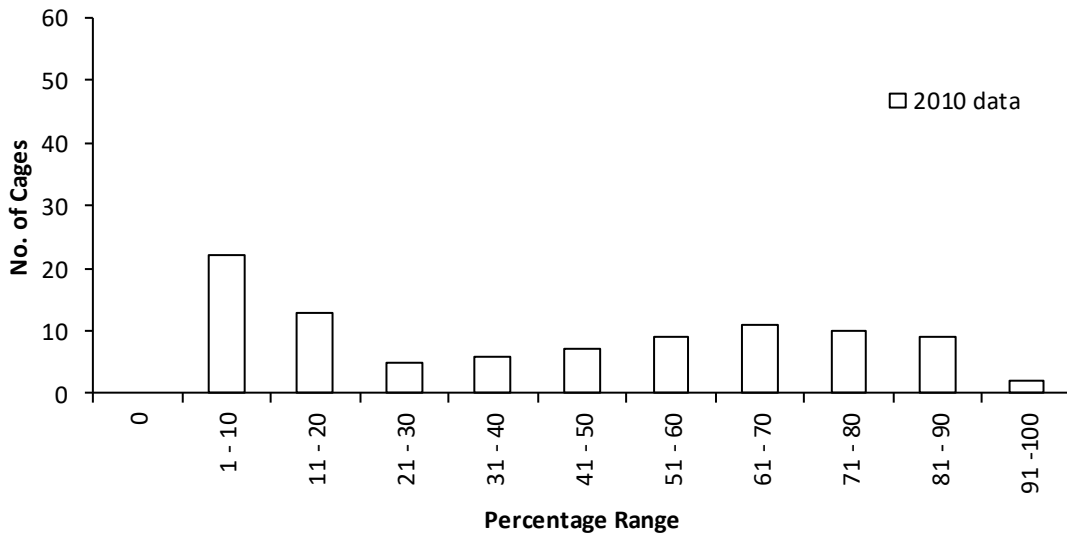


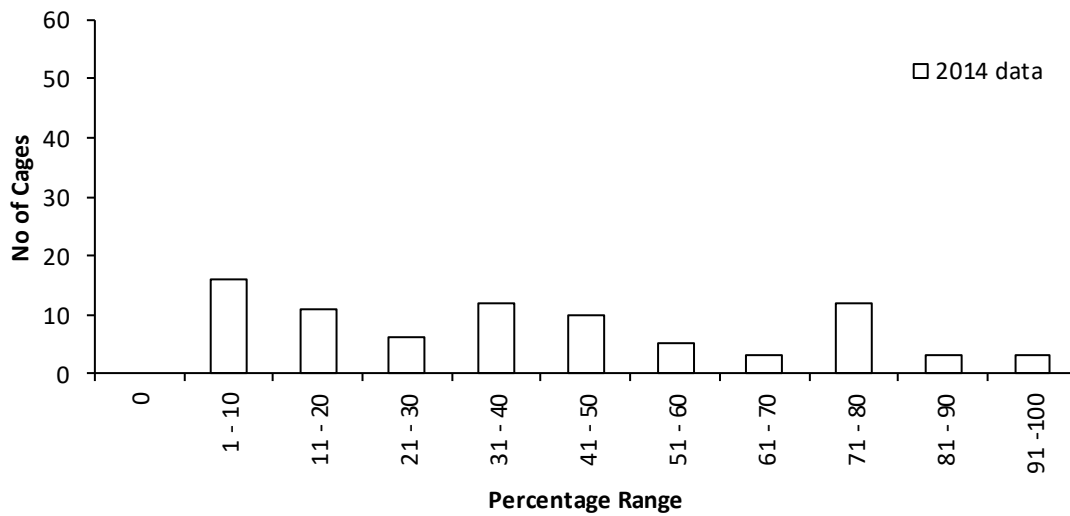
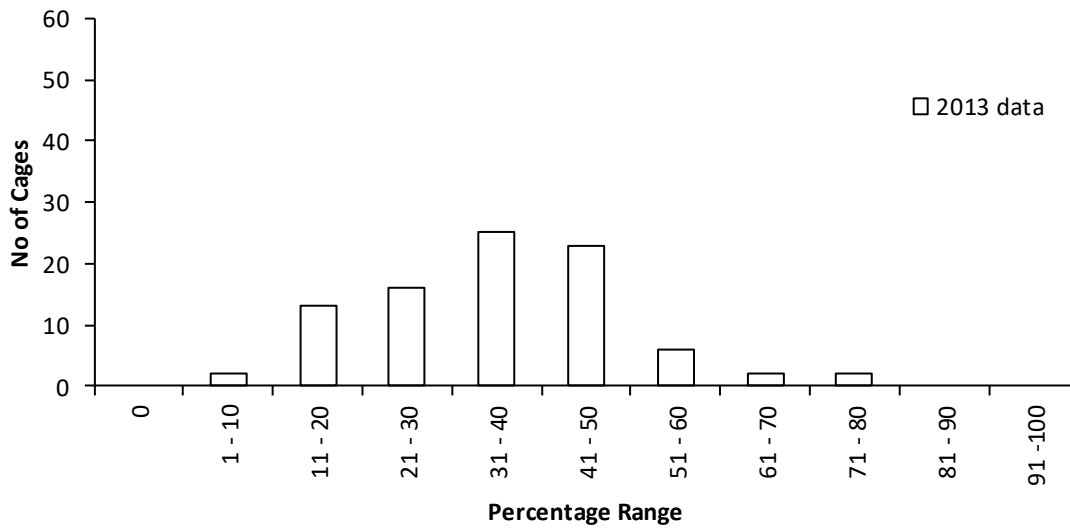
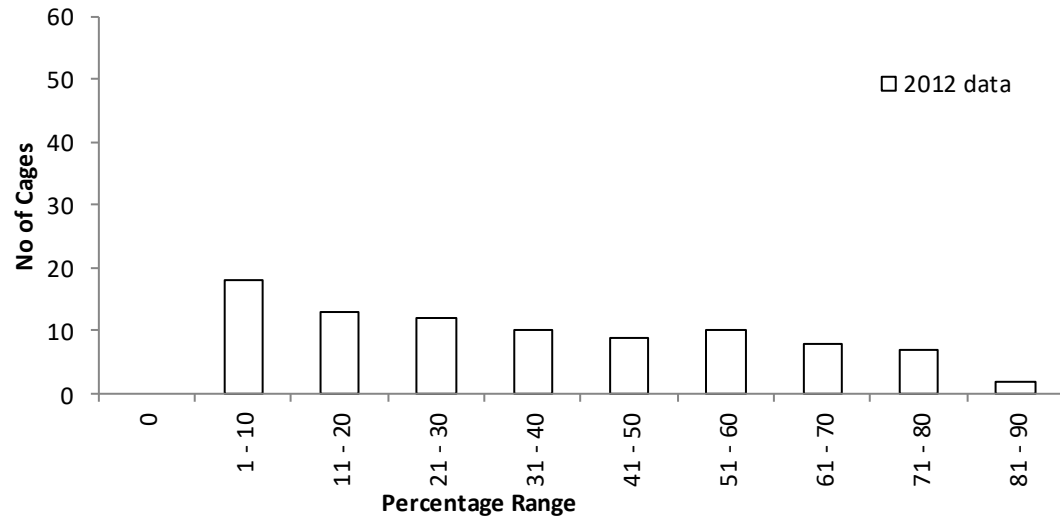
In the graph below the parasitism frequency at Vryheid increased with more cages producing higher parasitism, but the change was not as dramatic as at Linwood, since the two trend lines are almost parallel in the Vryheid depot graph, but at Linwood the trend lines converge at the higher percentage range from a very low number of cages producing low parasitism in 2007 at Linwood.



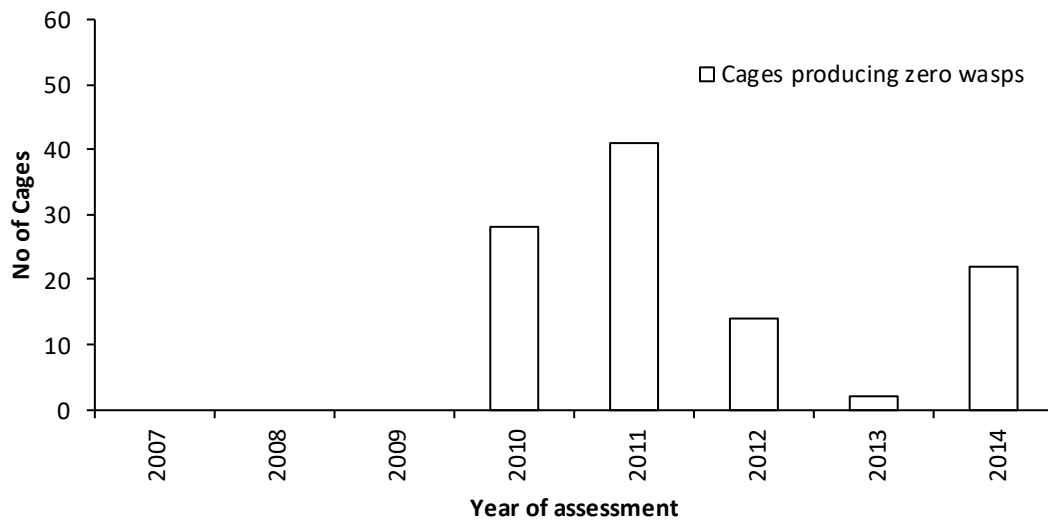
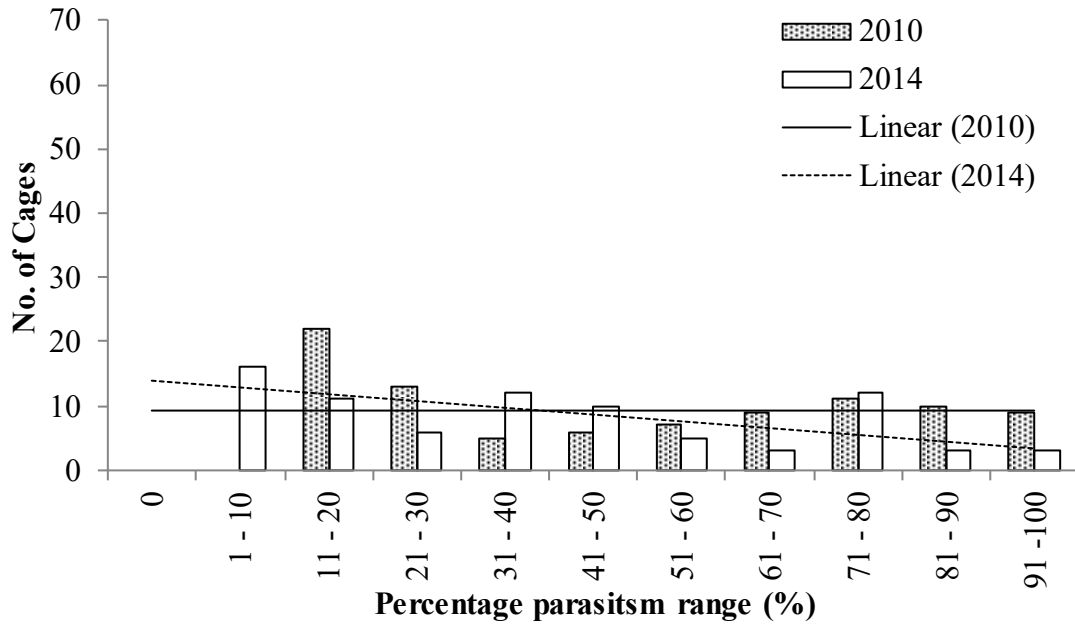


9.1.3. George depot data





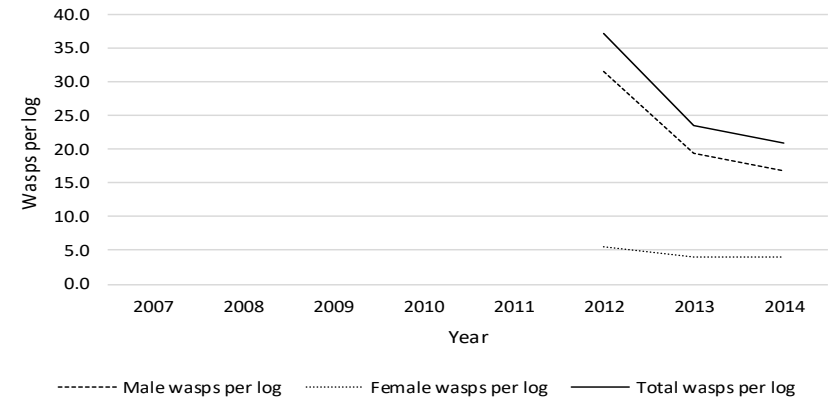
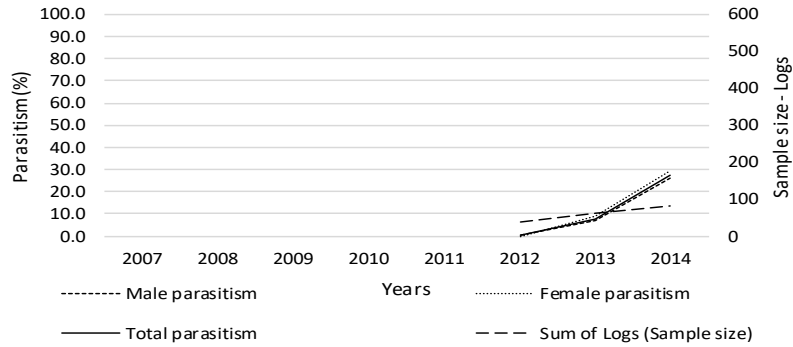
At the George depot, the parasitism percentage deteriorated over the years 2010 to 2014, and this can be attributed to the drought that affected the area in 2010 and then a more normal rainfall pattern returned in 2012 resulting in an even distribution of cages in all parasitism percentage ranges.



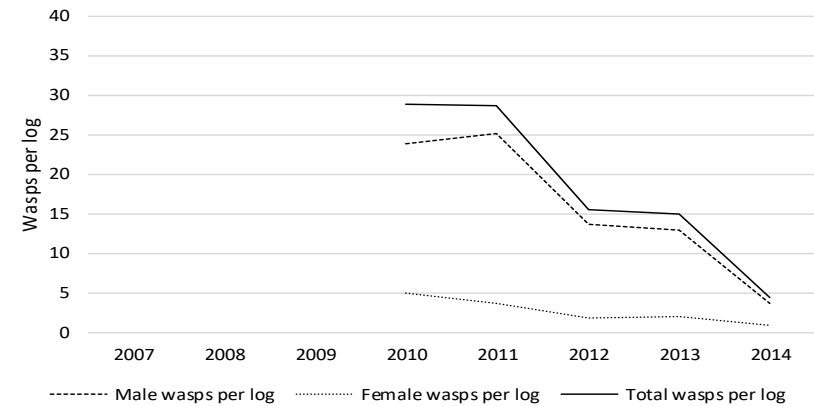
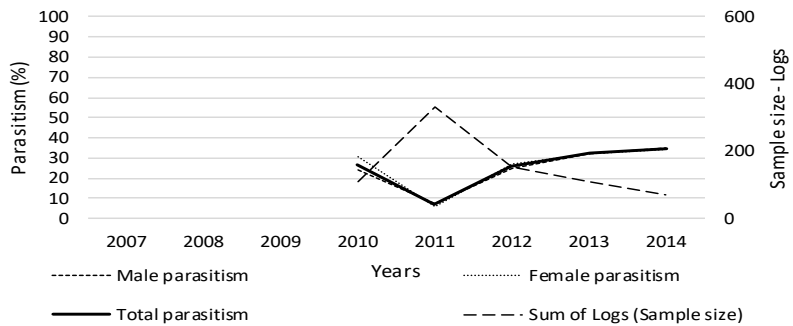
9.2. Appendix 2

The data below by region indicated the results that have been achieved through inoculation of *D. siricidicola*. The result was also evident in the *S. noctilio* wasps which emerged and were countered as wasps per log indicating a reduction in the *S. noctilio* population. For the forestry industry, this would indicate that fewer trees should have been attacked by *S. noctilio* in the following year.

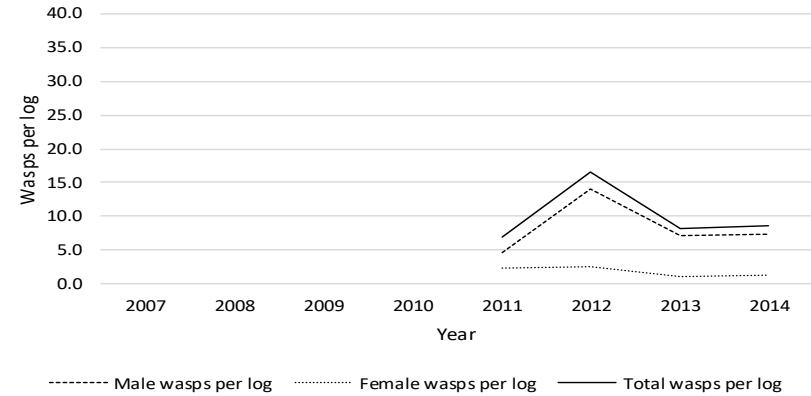
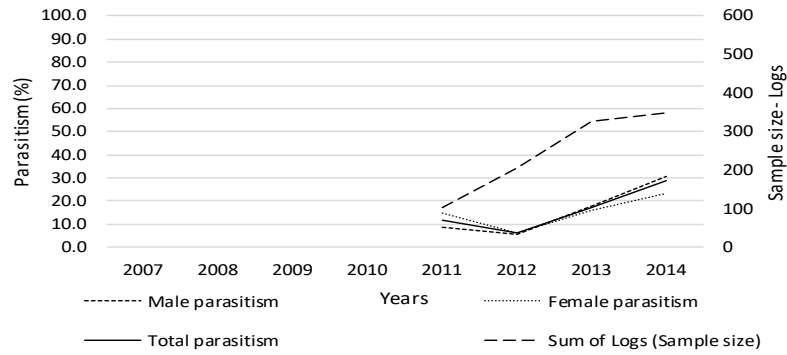
9.2.1. Limpopo region parasitism and wasp per log trends



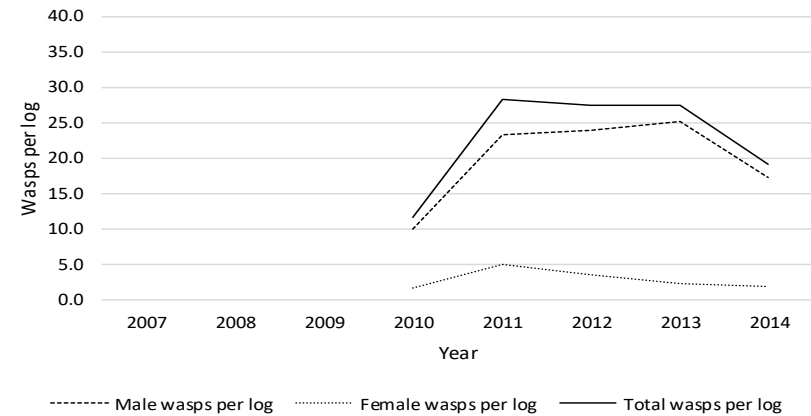
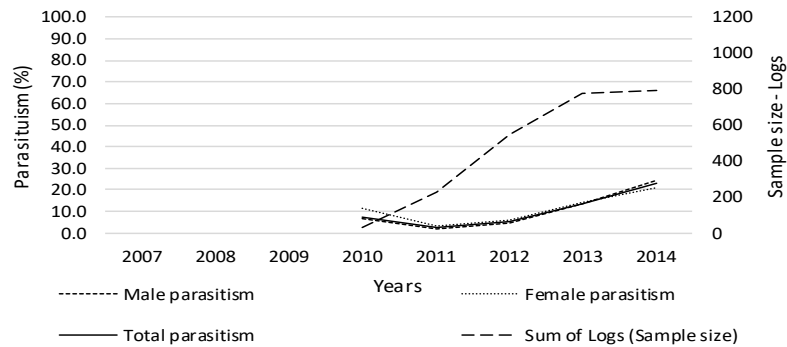
9.2.2. Central region parasitism and wasp per log trends



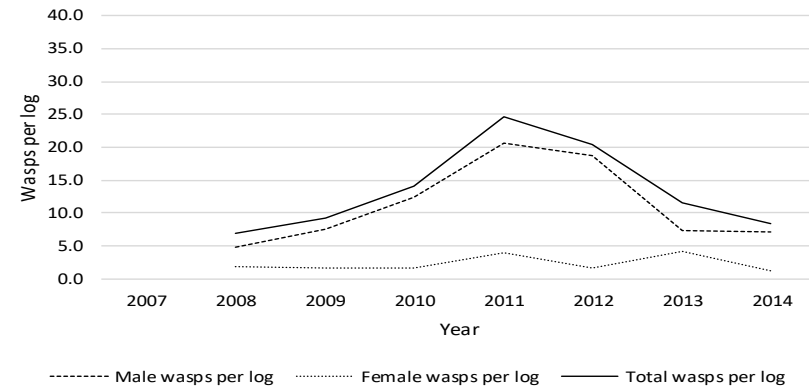
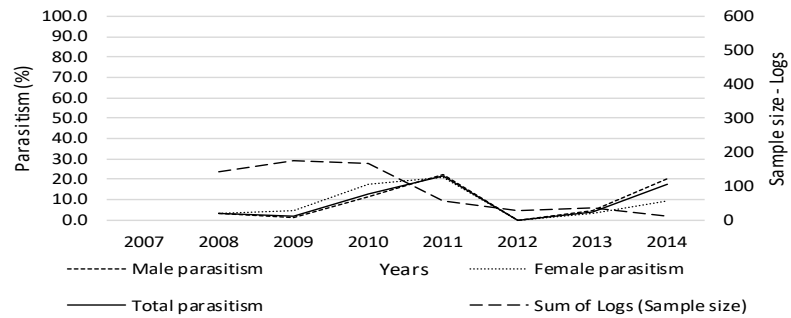
9.2.3. Mpumalanga North region parasitism and wasp per log trends



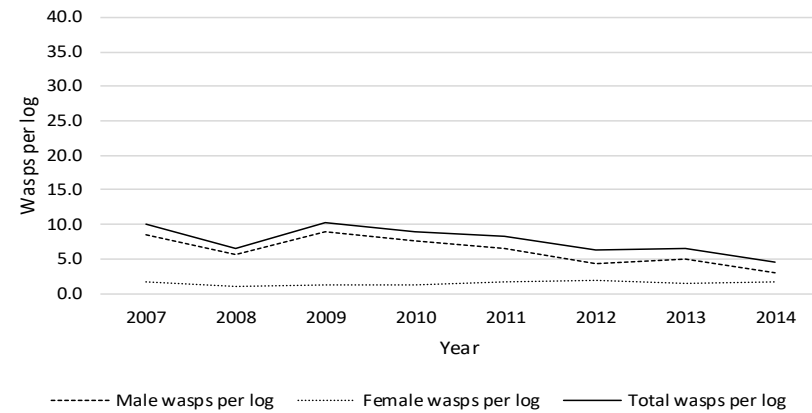
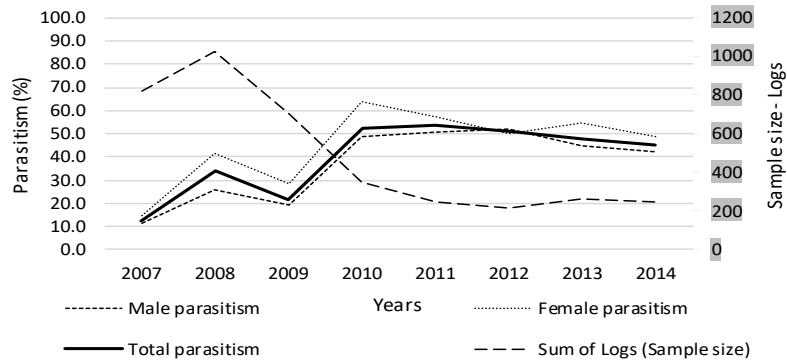
9.2.4. Mpumalanga South region parasitism and wasp per log trends



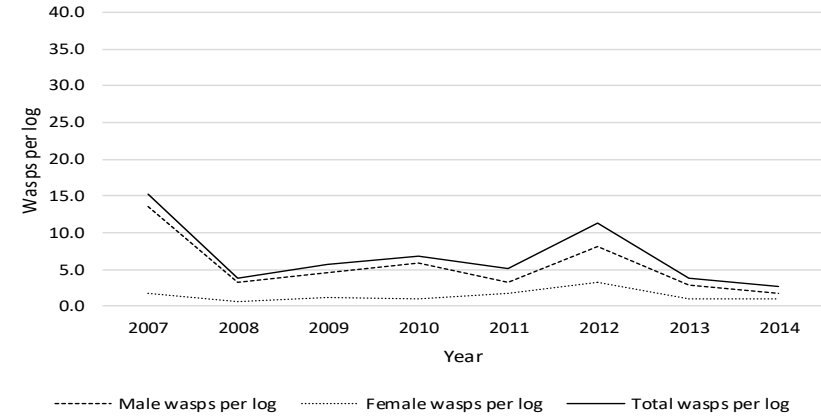
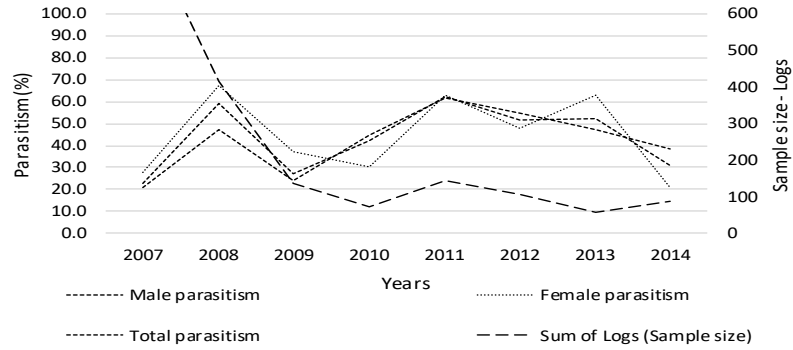
9.2.5. Northern KwaZulu-Natal region parasitism and wasp per log trends



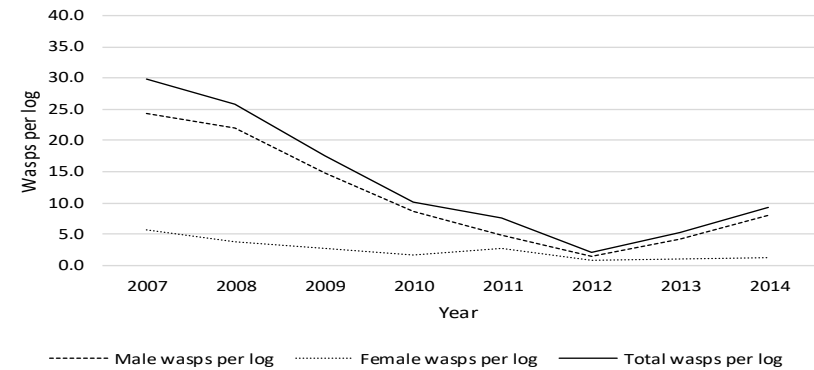
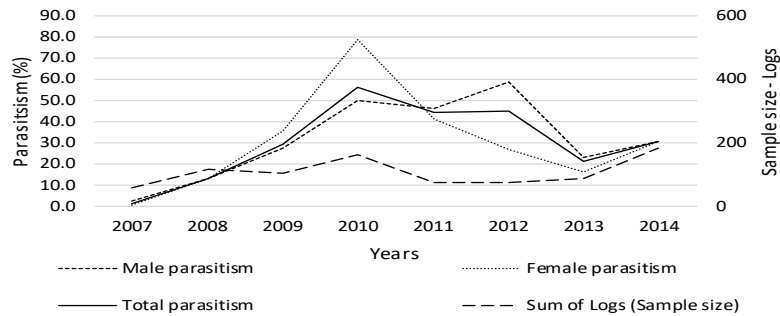
9.2.6. KwaZulu-Natal Midlands region parasitism and wasp per log trends



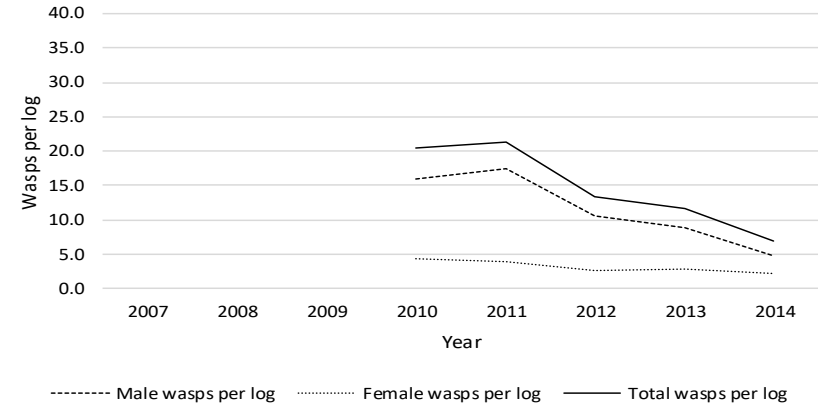
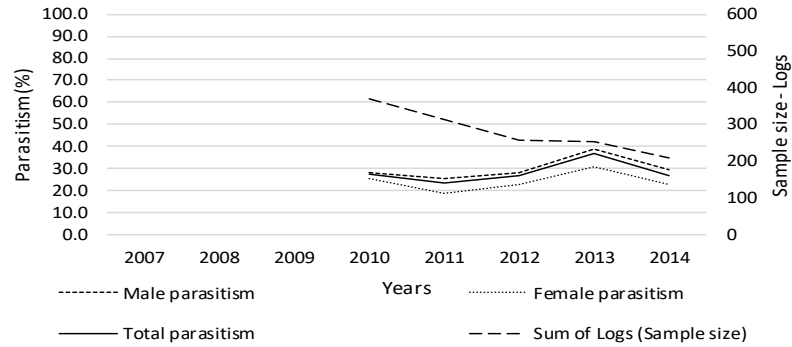
9.2.7. Southern KwaZulu-Natal region parasitism and wasp per log trends



9.2.8. Eastern Cape region parasitism and wasp per log trends



9.2.9. Southern Cape region parasitism and wasp per log trends



9.2.10 Western Cape region parasitism and wasp per log trends

