CHAPTER TWO

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

Published as: Hurley BP, Slippers B, Croft PK, Hatting HJ, van der Linde M, Morris AR, Dyer C, Wingfield MJ. 2008. Factors influencing parasitism of *Sirex noctilio* (Hymenoptera: Siricidae) by the nematode *Deladenus siricidcola* (Nematoda: Neotylenchidae) in summer rainfall areas of South Africa. Biological Control 45: 450-459.

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

Introduction

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

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

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

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

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

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

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

Materials and Methods

Sites

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

Inoculation with *D. siricidicola*

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

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

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

Moisture measurements of trees

The moisture content of the inoculated and control trees was measured with a Bes Bollmann moisture probe (model H D1 3.10, Gottmadingen, Germany). The probe was

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

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

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

oven-dry measurements were calculated as percentage moisture content equal to oven dry weight over fresh weight x 100.

Collection of billets

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

Dissection and measurement of emerging *S. noctilio* wasps

Wasps emerging from the drums were collected and transported to the laboratories of FABI, Pretoria, where they were dissected. Only female *S. noctilio* wasps are involved in moving *D. siricidicola* to freshly attacked trees, thus ensuring the survival of the nematodes. Consequently, female parasitism is viewed as the most accurate indication of the nematodes' influence on *S. noctilio* populations. Dissections, therefore, focused on female wasps, although a proportion of the male wasps were also dissected to ensure sufficient data for the

analyses. Wasps were dissected and the eggs of female wasps and the testes of male wasps examined for the presence of *D. siricidicola*, using light microscopy. The lengths of 2611 emerging wasps were measured from the tip of the head to the tip of the cornus (short dorsal spine at the apex of the abdomen).

Statistical analysis

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

Correlation and regression analyses were performed to examine the relationship between parasitism and moisture content. For these analyses the mean parasitism values over time per drum and the corresponding mean moisture content values over time were used. Correlation and regression analyses were also performed to examine the relationship between

moisture content values obtained with the moisture probe and with the oven-dry method. SAS version 8.2 (SAS Institute, 2001) was used for all statistical analyses.

Results

General

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

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

Correlation between moisture probe and oven-dry method

A Pearson's Correlation was used to test for the association between moisture content values obtained with the moisture probe and values obtained using the oven-dry method,

regarded as actual moisture content values. The association between the two methods was high (correlation coefficient = 0.89, $p < 0.0001$), providing confidence in the values obtained using the moisture probe. Furthermore, a regression analysis was done to determine the nature of this relationship (r-square = 0.79):

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

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

Moisture content over time

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

Site

Parasitism obtained from trees that were felled and inoculated was below 10 % for both sites (Table 2). There was no significant difference for total parasitism (from male and female wasps) between the sites ($F = 7.85$; $df = 2, 3660$; $P = 0.25$). There was a significant

difference in emergence numbers between the two sites, with Site 2 having significantly more males (F = 28.12; df = 2, 31584; P = 0.002), females (F = 28.12; df = 2, 31584; $P < 0.001$) and total wasps (F = 28.12; df = 2, 31584; $P < 0.001$) emerging on average than Site 1 (felled trees only). The sex-ratio was 1:10 females to males at Site 2 and 1:13 at Site 1. The average moisture content over time, from felled trees, was significantly higher at Site 1 (LS mean = 39.6 %) than at Site 2 (LS mean = 36 %) (F = 77.59; df = 1, 4200; $P < 0.0001$).

Nematode source and control

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

Inoculation time

Parasitism from trees inoculated in the first inoculation period (beginning March) was higher than that from the later two inoculation periods (Table 2). This difference in parasitism was significant when comparing total parasitism (F = 23.95; df = 2, 3660; $P < 0.001$), and

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

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

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

Tree section

For both felled and standing trees, parasitism was highest in the bottom section of the tree and lowest in the top section (Table 2). However, the ranking of parasitism between tree sections of felled trees differed between the two sites (Fig. 3). At Site 2, the highest parasitism was obtained from the bottom section, while at Site 1 the highest parasitism was obtained

from the middle section. For both sites, the lowest parasitism was obtained from the top section, and although this difference was not significant at Site 1 considering the Bonferroni correction, it was still considerable. As expected, parasitism from the middle and top sections of standing trees was very low, as only the bottom section was inoculated. The same ranking of parasitism between tree sections for the different sites was observed from control trees.

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

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

Influence of moisture content on parasitism

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

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

Size of emerging *S. noctilio*

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

Felled versus standing trees

Parasitism was higher in trees that were felled and inoculated as opposed to trees that were inoculated standing (Table 2). This was as expected, as only the bottom section of standing trees had been inoculated. When only the bottom sections of the trees were compared, parasitism remained higher in felled trees (Table 2), but this difference was not

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

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

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

Discussion

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

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

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

the virulence of the nematodes used in South Africa from 2004 to 2006 is unlikely to be the cause of the low parasitism obtained in the summer rainfall areas of the country.

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

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

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

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

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

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

Focusing inoculations where moisture content is adequate should increase inoculation success substantially in the summer rainfall regions of South Africa. Data from our study show that it is optimal to inoculate the bottom and middle sections of felled trees in the first inoculation period, with 15.1 % of the female *S. noctilio* from the entire tree being parasitized from inoculating these two sections (Table 4). If only the bottom sections are inoculated in the first inoculation period, 8.5 % of the female *S. noctilio* are parasitized. Inoculating the top

sections of felled trees increased the parasitized females by only 1.7 %, and is probably not worth the time and resources required for the inoculations. Inoculating standing trees limits the inoculations to the bottom third of the tree and would thus be considerably less effective than inoculating felled trees. However, the cost and safety of the different inoculation techniques would need to be considered when deciding on the most feasible approach.

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

The low levels of inoculation success using *D. siricidicola* in the summer rainfall region of South Africa, even when wood moisture content is adequate, questions whether it will be feasible to use this nematode as a biological control agent in this region. One positive indication was, however, that background parasitism was found at both sites. This shows that despite the low parasitism levels in these areas for the 2004 and 2005 inoculations, *D. siricidicola* has become established, albeit at very low levels. Furthermore, there had been no previous inoculations at Site 2, indicating that infected *S. noctilio* moved from surrounding areas that had been inoculated, and possibly from other plantations. These results are encouraging, as they show the potential for *D. siricidicola* to naturally spread despite low inoculation success. It remains to be determined whether the rate at which this might occur,

given low inoculation success, will be sufficient to reduce *S. noctilio* populations in this area to economically tolerable levels. Furthermore, the similarity in patterns of parasitism regarding time and tree section between inoculated and control trees suggest that the same barriers prevent high parasitism in both cases. The development of background parasitism at inoculated and un-inoculated sites requires further investigation to address these concerns.

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

References

Ahumda R. 2002. *Diseases in commercial Eucalyptus plantations in Chile, with special reference to Mycosphaerella and Botryosphaeria species.* M.Sc. thesis, Faculty of Natural and Agricultural Science, University of Pretoria, Pretoria, South Africa.

Bedding RA, Akhurst RJ. 1974. Use of the nematode *Deladenus siricidicola* in the biological control of *Sirex noctilio* in Australia. Journal of the Australian Entomological Society 13: 129-135.

Bedding RA, Akhurst RJ. 1978. Geographical distribution and host preferences of *Deladenus* species (Nematoda: Neotylenchidae) parasitic in siricid woodwasps and associated hymenopterous parasitoids. Nematologica 24: 286-294.

Bedding RA, Iede ET. 2005. Application of *Beddingia siricidicola* for Sirex woodwasp control. *Nematodes as Biocontrol Agents* (ed. by P.S. Grewal, R. Ehlers and D.I. Shapiro-Ilan), pp. 385-400. CAB International, Wallingford, U.K.

Carnegie AJ, Elderidge RH, Waterson DG. 2005. History and management of Sirex wood wasp in pine plantations in New South Wales, Australia. New Zealand Journal of Forestry Science 35: 3-24.

de Groot P. 2007. An overview of the *Sirex noctilio* status in Canada. In: International Sirex Symposium, 10-11 May 2007 (www.fabinet.up.ac.za/sirex/index). Pretoria, South Africa, pp. 17.

Gilbert JM, Miller LW. 1952. An outbreak of *Sirex noctilio* in Tasmania. Australian Journal of Biological Sciences 22: 905-914.

Haugen DA, Underdown MG. 1993. Reduced parasitism of *Sirex noctilio* in radiata pines inoculated with the nematode *Beddingia siricidicola* during 1974-1989. Australian Forestry 56: 45-48.

Haugen DA, Bedding RA, Underdown MG,. Neumann FG. 1990. *National strategy for control of Sirex noctilio in Australia.* Australian Forest Grower 13, No. 2.

Hoebeke ER, Haugen DA, Haack RA. 2005. *Sirex noctilio*: discovery of a Palearctic siricid woodwasp in New York. Newsletter of the Michigan Entomological Society 50: 24-25.

Hurley BP, Slippers B, Wingfield MJ. 2007a. A comparison of control results for the alien invasive woodwasp, *Sirex noctilio*, in the southern hemisphere. Agricultural and Forest Entomology 9: 159-171.

Hurley BP, Slippers B, Hatting HJ, Croft PK, Wingfield MJ. 2007b. The Sirex control program in the eastern parts of South Africa: Lessons from research efforts between 2004 - 2006. In: International Sirex Symposium, Pretoria, South Africa, 9-16 May, www.fabinet.up.ac.za/sirex/index (abstract only), p. 36.

Iede ET, Penteado SRC, Schaitza EG. 1998. *Sirex noctilio* problem in Brazil - detection, evaluation and control. *Proceedings of a conference: Training in the control of Sirex noctilio by use of natural enemies* (ed. by E. Iede, E. Shaitza, S. Penteado, R. Reardon and T. Murphy), pp. 45-52. USDA Forest Service (FHTET 98-13).

King JM. 1966. Some aspects of the biology of the fungal symbiont of *Sirex noctilio*. Australian Journal of Botany 14: 25-30.

Klasmer P, Fritz G, Corley J, Botto E. 1998. Current status of research on *Sirex noctilio* F. in the Andean-Patagonian region in Argentina. *Proceedings of a conference: Training in the control of Sirex noctilio by use of natural enemies* (ed. by E. Iede, E. Shaitza, S. Penteado, R. Reardon and T. Murphy), pp. 89-90. USDA Forest Service (FHTET 98-13).

Madden JL. 1981. Egg and larval development in the woodwasp, *Sirex noctilio* F. Australian Journal of Zoology 29: 493-506.

Maderni JFP. 1998. *Sirex noctilio* F.: present status in Uruguay. *Proceedings of a conference: Training in the control of Sirex noctilio by use of natural enemies* (ed. by E. Iede, E. Shaitza, S. Penteado, R. Reardon and T. Murphy), pp. 81-82. USDA Forest Service (FHTET 98-13).

Miller D, Clarke AF. 1935. *Sirex noctilio* (Hym.) and its parasites in New Zealand. Bulletin of Entomological Research 26: 149-154.

SAS Institute, 2001. Users Guide. SAS Institute Inc., Cary NC, USA.

Spradbery JP, Kirk AA. 1978. Aspects of the ecology of siricid woodwasps (Hymenoptera: Siricidae) in Europe, North Africa and Turkey with special reference to the biological control of *Sirex noctilio* F. in Australia. Bulletin of Entomological Research 68: 341-359.

Talbot PHB. 1977. The *Sirex-Amylostereum-Pinus* association. Annual Review of Phytopathology 15: 41-54.

Taylor KL. 1981. The Sirex woodwasp: ecology and control of an introduced forest insect. In: Kitching, R.L. and Jones, R.E (Eds.), The Ecology of Pests – some Australian Case Studies. CSIRO, Australia, pp. 231-248.

Tribe GD. 1995. The woodwasp *Sirex noctilio* Fabricius (Hymenoptera: Siricidae), a pest of *Pinus* species, now established in South Africa. African Entomology 3: 215-217.

Tribe GD, Cillié JJ. 2004. The spread of *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) in South African pine plantations and the introduction and establishment of its biological control agents. African Entomology 12: 9-17.

Zondag R. 1969. A nematode infection of *Sirex noctilio* (F.) in New Zealand. New Zealand Journal of Science 12: 732-747.

Zondag R. 1971. Control of *Sirex noctilio* (F.) with *Deladenus siricidicola* Bedding. Part I - 1967 Field trial. New Zealand Journal of Forestry Science 1: 5-14.

Zondag R. 1979. Control of *Sirex noctilio* (F.) with *Deladenus siricidicola* Bedding. Part II. Introductions and establishments in the South Island 1968-75. New Zealand Journal of Forestry Science 9: 68-76.

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

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

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

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

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

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

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

[a]Pearson's Correlation Coefficient

[b]p-value

[c]number of observations

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

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

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

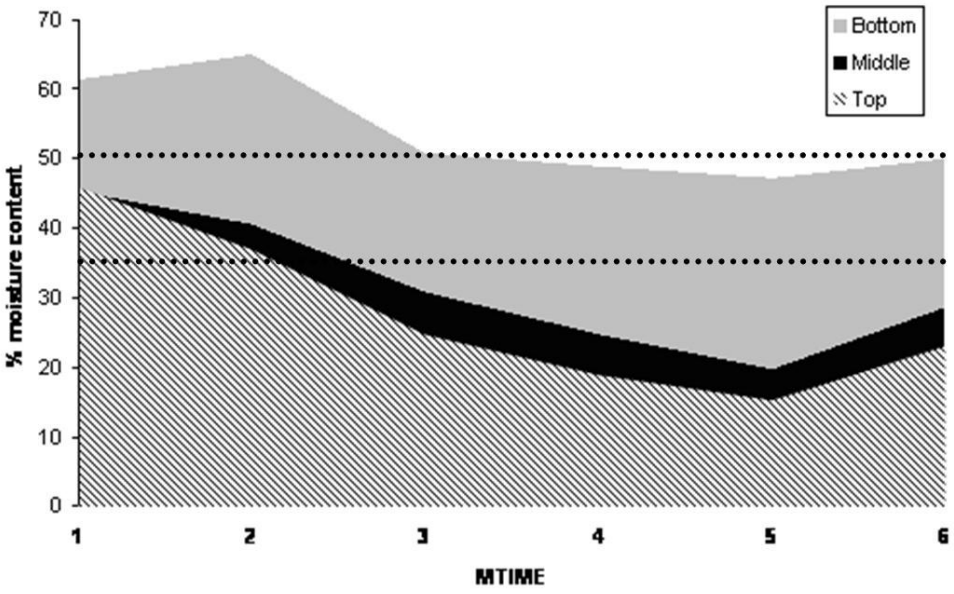

Bottom
Middle
Top
% moisture content
70
60
50
40
30
20
10
0
1
2
3
4
5
6
MTIME

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

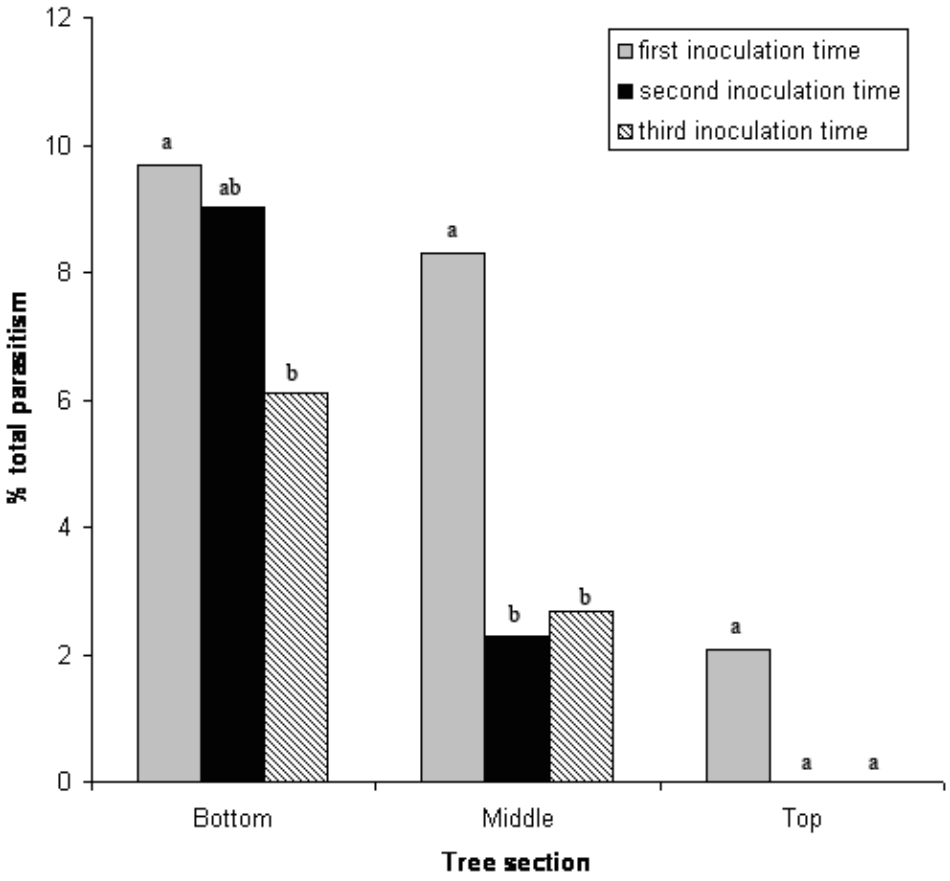

12
10
8
6
4
2
0
% total parasitism
first inoculation time
second inoculation time
third inoculation time
a
ab
b
a
b
b
a
a
a
Bottom
Middle
Top
Tree section

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

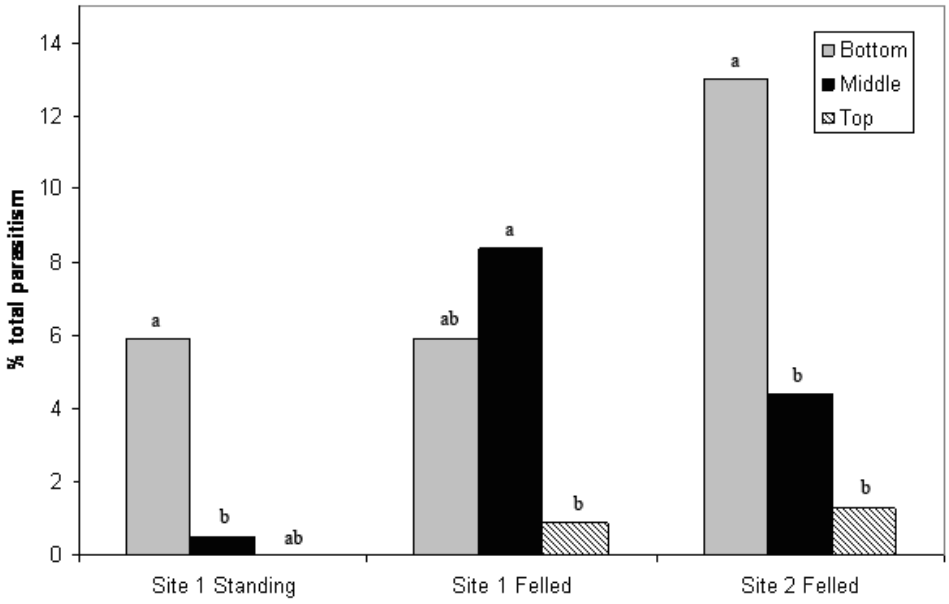

% total parasitism
14
12
10
8
6
4
2
0
Bottom
Middle
Top
a
b
ab
a
ab
b
a
b
b
Site 1 Standing
Site 1 Felled
Site 2 Felled

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

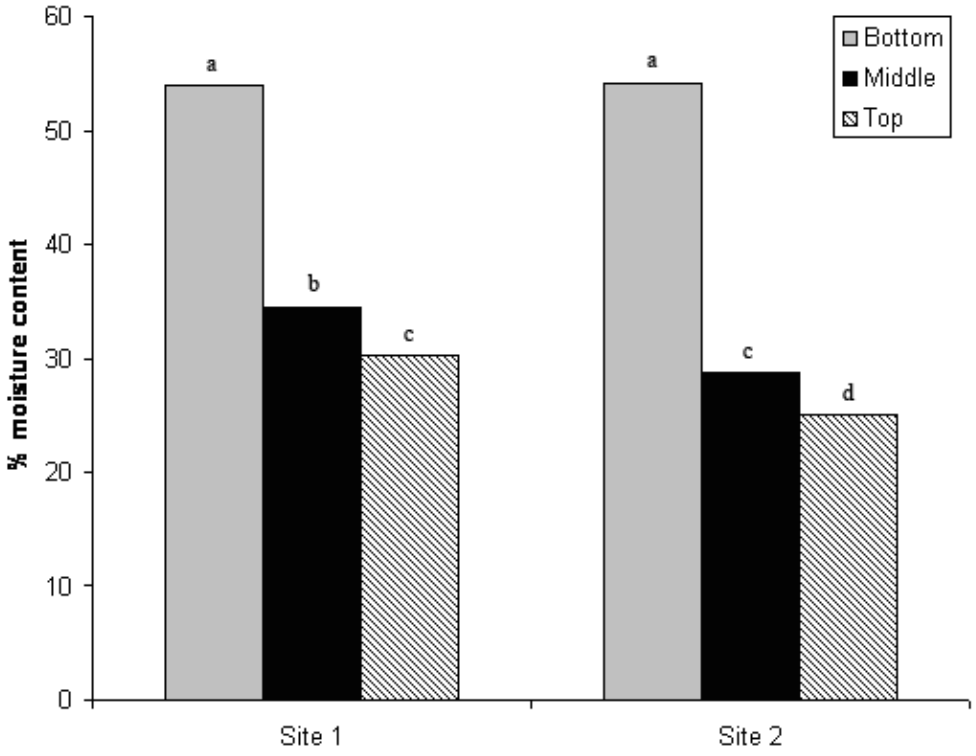
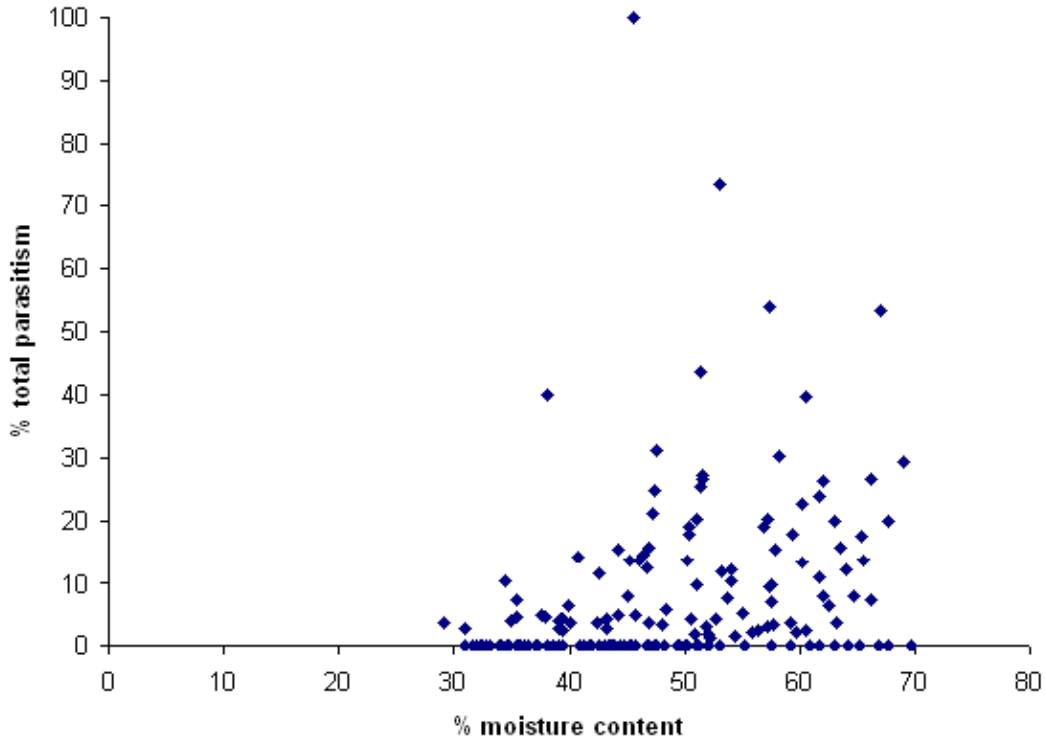

% moisture content
60
50
40
30
20
10
0
a
b
c
a
c
d
Bottom
Middle
Top
Site 1
Site 2

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


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