REFEREED PAPER

MONITORING WHITE GRUB BEETLE LARVAE (SCARABAEIDAE) IN SUGARCANE IN THE MIDLANDS NORTH REGION OF KWAZULU-NATAL

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Abstract

Scarabaeid beetle larvae (Coleoptera: Scarabaeidae) are common soil pests throughout the South African sugarcane industry. From 2001 to 2010, with the exception of 2002, abundance and distribution of *Schizonycha affinis* and *Hypopholis sommeri* (Melolonthinae) were monitored in the Midlands North region of KwaZulu-Natal. Average annual infestation was 2.11±0.60 larvae per 30 cm^3 pit. Lowest infestation in 2008 was 0.66 larvae/pit and the highest was 6.19 larvae/pit in 2009. Ecozones (EZs) 3, 4 and 7 had the highest infestations (2.11±0.08 larvae/pit), moderate infestations were recorded in EZs 1, 5, 6 and 10 (1.38±0.05 larvae/pit). Lower infestations occurred in EZs 2, 8 and 9 (0.79±0.06 larvae/pit) with the lowest levels recorded in EZ 11 (0.27±0.04 larvae/pit). Plant to sixth ratoon crops had progressively higher infestations (r=0.86). Fields between 25 to 35 ha had significantly higher larval counts compared with smaller fields. As an overall trend varieties N37, N11, N12, N16, N31, N21 and N27 had relatively high larval counts (1.56±0.07 larvae/pit) compared with N25, N36, N26 and N29 (0.29±0.07 larvae/pit). However, the count was significantly higher only for N37. Regular monitoring by the Local Pest, Disease and Variety Control Committee (LPD&VCC) in the Midlands North region provided data which show overall that beetle larvae persist in this region. Ongoing research into control options within an integrated pest management approach is thus warranted.

Keywords: white grub, Scarabaeidae; sugarcane, abundance, distribution

Introduction

Sugarcane in the South African industry is attacked by various scarabaeid beetles (Coleoptera: Scarabeidae). These insects are generally referred to as white grubs. Second and third stage larvae feed on and damage roots, causing retarded growth and occasionally total crop failure; moreover, crops with severely damaged roots lodge during strong winds

(Carnegie, 1988). McArthur and Leslie (2004) indicated the extent of losses that this group of pests causes to sugarcane in the Midlands region of the industry as an average reduction in yield of between 23 and 55 tons cane/ha depending on the variety and the season.

Of the 13 scarabaeid beetle taxa recovered in the South African sugarcane industry, *Schizonycha affinis* Boh. and *Hypopholis sommeri* Burm. (Scarabaeidae: Melolonthinae) are the most prevalent in the Midlands North region of KwaZulu-Natal (Way, 1997). Larvae of these species were monitored in this region over the past 10 years by the Local Pest, Disease and Variety Control Committee (LPD&VCC) and this programme produced the data presented in this paper on abundance, distribution and agronomic parameters influencing these insect pests.

Materials and Methods

Over several years the LPD&VCC in the Midlands North mill region of KwaZulu-Natal has surveyed for beetle larvae. For this study the data gathered from 2001 to 2010, excluding 2002 due to logistical reasons, were analysed to obtain an overview of the current situation with regard to these pests.

The sampling procedure comprised conducting standard surveys from June to September, when larvae are at their largest stage. Where possible, the same commercial sugarcane fields on the same farms were surveyed each year, for valid comparison. The extent of the survey programme varied each year according to practicalities as shown in Appendix Table 1. However, each year the EZs were evenly represented. As a general rule six fields were sampled on at least four farms in different EZs. In all instances five pits were sampled in each field. The pits of 30 cm^3 were dug under sugarcane stools. From the excavated soil and plant material from the pits, larvae and other life stages were carefully collected for identification and counting. The pits were sampled in a zig-zag transect 10 m along the row and 5 rows apart. Raster patterns on the dorsal surface of the last larval abdominal segment were used to identify the species collected (Sweeny, 1967; Dittrich-Schröder *et al.*, 2009) *Schizonycha affinis* has a horse-shoe shaped raster pattern, and characteristically *H. sommeri* has no distinct raster pattern. Larval identifications were done in the field using a 10x hand lens.

In this paper, data from the 11 EZs in the Midlands North region, as shown in Figure 1 and Table 1 given by Webster *et al*. (2009), were analysed separately.

Survey information recorded on field size and cane varieties were used to investigate possible relationships between these agronomic factors and larval counts. In addition, the larval count data gathered from EZ 4 were analysed to investigate the distribution of individuals within pits. The latter analysis involved determining the number of pits in the database containing zero, one, two and so on, up to 10 individuals. This value was expressed as a percentage of the total number of pits surveyed and was then used to present the cumulative percentage of pits with progressively increasing numbers of larvae per pit.

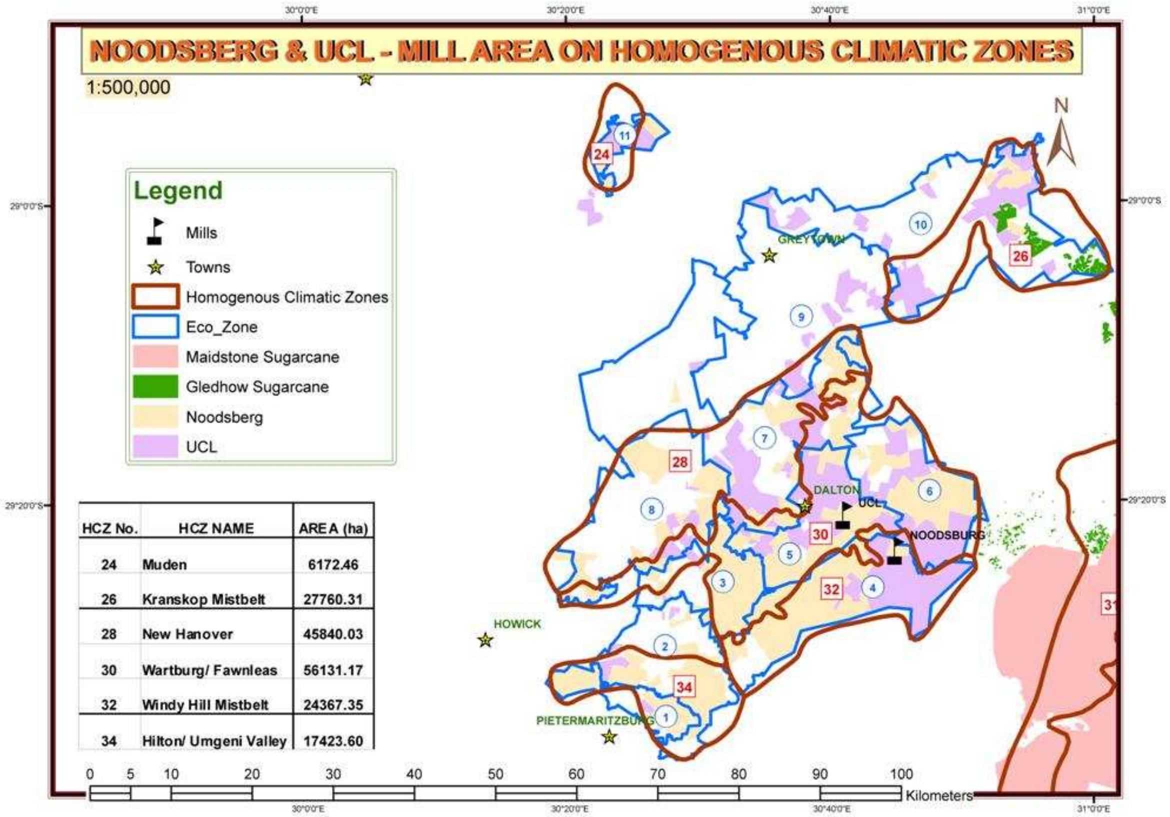

Figure 1. Ecozone localities in the Midlands North mill region.

Table 1. Altitude and main cliamatic features of the ecozones in the Midlands North region (from Webster *et al*., 2009).

Ecozone	Rainfall range (mm)	Altitude range (m)	Maximum temperature (°C)	Minimum temperature (°C)
1	801-850	541-900	26.7	6.1
2	701-750	451-900	27.5	5.9
3	751-750	451-900	26.8	5.8
4	901-1100	901-1400	25.3	7.0
5	801-850	901-1400	25.7	6.0
6	751-800	451-1400	26.6	6.8
7	851-900	901-1400	25.7	5.3 (frost)
8	901-1100	451-1400	27.1	5.3 (frost)
9	751-850	901-1400	26.1	4.6 (frost)
10	901-1100	901-1400	25.4	6.5
11	651-700	451-900	28.6	4.3 (frost)

Soil samples were taken from two randomly selected farms in EZ 4 (representing high mean larval counts) and EZ 8 (representing low mean larval counts) to investigate the possible link between soil texture (proportion of sand, silt and clay, expressed as a percentage) and larval abundance. These soil samples were taken using a standard soil augur. The samples were sent to the South African Sugarcane Research Institute (SASRI) where the Fertiliser Advisory Service (FAS) performed appropriate determinations to measure the soil texture. This work involved using the Bouyoucos hydrometer, which operates according to Stokes law.

The following protocol was followed in the FAS laboratory to determine texture: 48 g of dried and sieved (1 mm) sample was saturated with 150 ml Calgon solution (sodium hydroxide and sodium hexametaphosphate solution) to which a few drops of silicon were added as an antifoaming agent. The samples were capped, and shaken in a flat bed shaker for 50 min at 250 rpm. Samples were then transferred into calibrated clay cylinders in which they were rinsed with water to the level of a calibrated mark. Each cylinder was sealed with a stopper, and mixed by shaking by hand for 30 seconds. To obtain silt and clay determinations, hydrometer readings were taken at 4 minutes and 2 hours, respectively. The proportion of clay was calculated as a percentage using the formula: the 2 h hydrometer value minus the blank value. The silt content was calculated using the formula: the 4 min value minus the 2 h value. The proportion of sand was calculated using the formula: 100 minus (clay % + silt %).

The larval count data had a negative binomial distribution, and were analysed using the Generalised Linear Mixed Effects Model (GLMM) procedure with a logratio-link function. Statistical significance for main effects and interactions were established using the F statistic. The Holm-Sidak test was used on the transformed means to quantify significant differences. For clarity the interpretation of raw means are presented. The soil texture data were analysed using a standard student T-test.

Results

Infestation

Scarabaeid larvae were consistently collected over the whole area throughout the years sampled. Average annual infestation was 2.11±0.60 larvae per 30 cm^3 pit. The lowest infestation of 0.66 larvae/pit occurred in 2008, and the highest was 6.19 larvae/pit in 2009. Significantly more larvae were recovered during 2009 (Table 2). Distribution across ecozones also varied through the years (Table 3). Most individuals were consistently recovered from EZ 4 (32.97% of all individuals collected over the nine year study period). Distribution within this EZ was erratic though, with 43% of the pits (stools) having no larvae, and 44% having 1 to 5 individuals. The remainder of the pits contained 6 or more individuals (Table 4). EZs 3, 4 and 7 had significantly higher infestations (2.11±0.08 larvae/pit) compared to EZs 2, 8 and 9 (0.79±0.06 larvae/pit), while EZ 11 was significantly least infested (0.27±0.04 larvae/pit) (Table 3).

Table 2. Scarabaeid larval infestation recorded from 2001 to 2010, excluding 2002, over the whole of the Midlands North mill region.

Year	Mean absolute estimate larvae/pit	Range (min-max)	N
2001	2.62d	0-26	1075
2003	1.94c	0-23	1140
2004	1.11b	0-19	840
2005	1.46c	0-26	1055
2006	0.71ab	0-13	1250
2007	0.84ab	0-13	445
2008	0.66a	0-22	425
2009	6.19e	0-54	278
2010	3.44d	0-45	284

Values followed by same letter in the same column are not significantly different ($F_{(8,6595)}$= 59.78, <0.001).

Table 3. Scarabaeid larval distribution, expressed as larva per pit by year in each Ecozone in the Midlands North mill region. Data obtained from the number of farms and the number of pits per region per year shown in Appendix Table 1.

Survey year	Ecozone in the Midlands North mill region										
	1	2	3	4	5	6	7	8	9	10	11
2001	1.88	-	1.10	5.49	2.76	2.16	1.81	0.65	-	7.12	-
2003	0.63	0.67	1.92	2.77	1.98	1.41	2.69	0.67	-	1.60	0.22
2004	-	-	1.87	1.24	0.96	-	0.91	-	-	0.80	-
2005	-	0.37	0.37	3.49	0.97	0.87	1.62	0.38	-	1.97	0.37
2006	0.93	0.33	0.07	0.90	0.64	0.91	0.80	0.52	-	0.84	0.33
2007	0.68	0.42	0.97	1.52	0.92	1.38	0.61	0.40	0.23	0.95	0.10
2008	-	0.48	1.53	0.99	0.73	0.58	0.83	0.55	0.07	0.47	0.05
2009	1.42	3.74	6.56	9.13	6.13	3.67	8.74	4.14	6.00	4.94	1.00
2010	4.72	2.17	3.11	5.52	2.63	3.92	3.60	2.13	1.50	2.08	1.08
Mean absolute estimate larvae/pit	1.35 bc	0.77 b	1.70 c	2.43 c	1.36 bc	1.28 bc	1.81 c	0.80 b	0.75 b	1.50 bc	0.27 a
Maximum larvae/pit	25	15	52	54	22	23	32	37	14	21	4
Pits sampled	175	407	261	1408	1083	569	1139	699	72	685	294

Dash indicates no data. Values followed by same letter in the same row are not significantly different (Wald statistic, $F_{(10,6785)}$ =11.26, <0.001)

Table 4. Scarabaeid larval infestation in Ecozone 4 assessed as the frequency of pits with increasing numbers of individuals, showing the cumulative percentage calculated from the total number of pits surveyed.

White grub infestation category (represented by the number of larvae recovered per pit)	Frequency of pits per category	% pits in each infestation category	Cumulative % of all the pits surveyed
0	608	43	43.0
1	231	16	59.4
2	171	12	71.6
3	95	7	78.3
4	76	5	83.7
5	57	4	87.7
6	33	2	90.1
7	28	2	92.1
8	18	1	93.4
9	12	1	94.2
10	17	1	95.4
	62*	4.4	

*More than 10 larvae/pit (outliers) omitted from the analysis

Effect of field size, crop age (ratoon), topographical aspect, cane variety and soil texture
Significantly more larvae were recovered from larger fields (between 25 and 35 ha) than from smaller fields (Table 5). Larval numbers increased in older crops, which strongly correlated with increasing ratoon age (from plant to sixth ratoon; r=0.863) (Table 6). Significantly higher infestations were found in flat fields, and the westerly facing fields compared with the south-west facing fields (Table 7).

Larval numbers could be linked to varieties planted in infested fields. It was possible to identify varieties commonly found in fields with relatively high larval numbers (N37, N11, N12, N16, N31, N21 and N27 = average 1.56±0.07 larvae/pit) and those commonly found in fields with a lower category of infestation (N25, N36, N26 and N29 = average 0.29±0.07 larvae/pit (Table 8). However, only the results from N37 were significantly different.

A high sand content was measured in soil samples taken from EZ 4, where larval infestations were generally the highest (Table 9 and appendix Table 2). In contrast, high clay content was measured in EZ 8 where few larvae were recovered.

Table 5. The relationship between scarabaeid larval infestation and fields of increasing area in the Midlands North mill region.

Field size (ha) category	Mean absolute estimate larvae/pit	N
0.0-4.9	1.72ab	2203
5.0- 9.9.0	1.38a	3747
10.0-14.9	1.70ab	492
15.0-24.9	1.54a	270
25.0-35.0	2.38b	80

Values followed by same letter in the same column are not significantly different ($F_{(10,6785)}$ = 5.7, <0.001)

Table 6. The relationship between scarabaeid larval infestations and ratoon age in the Midlands North mill region (0=plant cane, 1-12=increasing ratoon age).

Sugarcane ratoon age	Mean absolute estimate larvae/pit	N
0	0.98a	451
1	1.25ab	544
2	1.21ab	908
3	1.42ab	1323
4	1.34ab	1110
5	1.66ab	629
6	1.62ab	365
7	1.15ab	260
8	0.99a	126
9	0.97a	75
10	1.04ab	25
11	4.60b	5
12	1.27b	15

Values followed by same letter in the same column are not significantly different ($F_{(8.5159)}$ = 3.2, p<0.001)

Table 7. Comparison of scarabaeid larval infestations in fields with differing topographical aspects in the Midlands North mill region.

Field aspect	Mean absolute estimate larvae/pit	N	Predicted mean (Holm-Sidak (5%))
Flat	2.13	565	-0.48b
West	1.88	316	-0.53b
North-west	1.57	494	-0.59ab
South-east	1.56	590	-0.55ab
North	1.54	718	-0.61ab
North-east	1.53	699	-0.59ab
South	1.32	724	-0.58ab
East	1.28	320	-0.59ab
South-west	1.01	744	-0.68a

Values followed by same letter in the same column are not significantly different ($F_{(13.5796)}$ = 17.47, p<0.001)

Table 8. Relationship between scarabaeid larval infestations and sugarcane varieties cultivated in the same fields in the Midlands North mill region.

Variety	Mean absolute estimate larvae/pit	N
N37	1.76b	234
N11	1.68ab	68
N12	1.63ab	3684
N16	1.60ab	1675
N31	1.58ab	379
N21	1.48ab	82
N27	1.18ab	38
N25	0.47ab	62
N36	0.31ab	164
N26	0.20ab	45
N29	0.18ab	60

Values followed by same letter in the same column are not significantly different (Wald statistic, $F_{(10,6785)}$ = 3.09, p<0.001)

Table 9. Comparison of the soil textures of sugarcane fields with scarabaeid larvae between Ecozones 4 and 8 in the Midlands North region.

Variable	Ecozone 4		Ecozone 8		Test t-statistic, (p<0.001, 22 df)
	Mean	Standard error	Mean	Standard error	
Sand	62.08	2.52	25.08	0.97	13.72
Silt	6.33	0.41	16.08	0.753	-11.34
Clay	31.58	2.22	57.58	1.40	-9.93
Organic matter	3.14	0.17	4.71	0.22	-5.64

Discussion

During the nine year monitoring period scarabaeid larvae were recovered from sugarcane stools across the Midlands North mill region of KwaZulu-Natal. Infestation levels were similar to the levels recorded previously (Way, 1997), confirming the presence of these pests in numbers warranting control action. This survey information shows that larvae are more abundant in certain ecozones. This patchy distribution was reported previously (Way, 1997), and indicates that knowledge of this system is needed to better understand the interactions.

Carnegie (1988) suggested that the presence of wattle plantations influenced the numbers and distribution of scarabaeid larvae in the Midlands region. It is known that scarabaeid adults roost and mate in these trees. He also implicated high potential soils, and/or high rainfall. Further insights into the possible causes of infestation have been provided by this study which showed that larger fields, and older ratoons, supported higher numbers of larvae. It is suggested that larger fields of older ratoons by logic provide more stable habitat since the root environment remains untouched, despite repeated harvests of the above-ground biomass of the sugarcane crop. Mugalula *et al.* (2006) propose and provide evidence that ploughing out and harrowing fields to desiccate scarabaeid larvae is an effective cultural control measure, as the larvae are particularly susceptible to desiccation when the crop is ploughed-out at replant.

All the data used in this study was available from previous records. This illustrates the value of routinely collected data that is consistently and accurately recorded, and then scientifically analysed. Provided the data is ultimately treated in this manner, it is possible to obtain maximum benefit from these types of area-wide surveys. However, greater consistency, in terms of numbers of surveys and systematic coverage of the region is essential to allow more accurate and definitive conclusions. Nevertheless, these data have provided valuable insights and they represent baseline data from which important aspects of scarabaeid larval biology can be investigated in more detail in the Midlands North region. These additional aspects of research can easily be included within future scarabaeid larva surveying programmes conducted by the Midlands North LPD&VCC.

Furthermore, the synthesised information presented in this paper can lead to more efficient collections of target species for laboratory studies, and can be used also to site field trials. This study demonstrates that already available agronomic and environmental factors can be ‘superimposed’ on the scarabaeid surveys to refine control options when they are developed. Accurate identification of the surveyed species is an important first step required for monitoring programmes, particularly when there are suites of species involved (Way and du Toit, 1996; Allsopp, 2010). Once the taxonomy is resolved, it will be possible to refine this knowledge based approach to scarabaeid control in sugarcane fields showcased in this paper. It is pleasing to report that such studies have been completed (Sweeny, 1967) and have recently been implemented in the South African industry (Dittrich-Schröder *et al.*, 2009; Harrison, 2009).

This paper highlights the importance of continuously collecting data on white grubs and, indeed, all aspects of the sugarcane industry over a long period. An improvement in field surveys based on the use of such data will result in better pest and crop management.

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REFERENCES

Allsopp P (2010). Integrated management of sugarcane whitegrubs in Australia: An evolving success. *Ann Rev Entomol* 55: 329-49.

Carnegie AJM (1988). White grubs (Scarabaeoidea) continue to cause sporadic damage to sugarcane in South Africa and Swaziland. *Proc S Afr Sug Technol Ass* 62: 161-163.

Dittrich-Schröder G, Conlong DE, Way MJ, Harrison J du G and Mitchell A (2009). Identification key to scarabaeid beetle larvae attacking sugarcane in South Africa using DNA barcoding and integrative taxonomy. *Proc S Afr Sug Technol Ass* 82: 500-524.

Harrison J du G (2009). A taxonomic revision of the African leaf chafer genus *Asthenopholis* Brenske, 1898 (Coleoptera: Scarabaeidae: Melolonthinae): a SEM study. *Zootaxa* 2225: 1-48. ISSN 1175-5334 (online edition).

McArthur DG and Leslie GW (2004). Preliminary observations on the impact of whitegrub on sugarcane yields in the Midlands North region of the South African sugar industry. *Proc S Afr Sug Technol Ass* 78: 283-286.

Mugalula A, Tibakanya G and Conlong DE (2006). Recent developments in the control of *Idaecamenta eugeniae* (Coleoptera: Scarabaeidae: Melolonthinae) at Kinyara Sugar Works Ltd, Uganda. *Proc S Afr Sug Technol Ass* 80: 256-263.

Sweeny C (1967). The Scarabaeoidea associated with sugarcane in Swaziland, an account of preliminary investigations into the bionomics and control. August 1956 - June 1967. Ministry of Agriculture. Research Bulletin No. 16.

Way MJ (1997). Surveying white grub larvae (Scarabaeidae) in the South African Sugar Industry. *Proc S Afr Sug Technol Ass* 71: 95.

Way MJ and du Toit HA (1996). White grub working group: pest status, biology and rearing of white grub (Coleoptera: Scarabaeidae) pests in southern Africa. 6-7 June 1996. SASRI Technical Report, Mount Edgecombe, KwaZulu-Natal, 23 pp.

Webster TM, Brenchley PG and Conlong DE (2009). Progress of the area-wide integrated pest management plan for *Eldana saccharina* Walker (Lepidoptera: Pyralidae) in the Midlands North region of KwaZulu-Natal. *Proc S Afr Sug Technol Ass* 82: 471-485.

APPENDIX

Table 1. Numbers of surveys for scarabaeid larvae conducted in the Midlands North region of the South African sugarcane industry.

Year	Zone	1	2	3	4	5	6	7	8	9	10	11
2001	Farms	1	0	1	2	3	2	2	2	0	1	0
	Fields	5	0	6	11	16	10	21	11	0	5	0
	Pits	25	0	30	55	80	50	105	55	0	25	0
2003	Farms	1	3	1	11	7	5	5	3	0	4	0
	Fields	6	18	6	66	41	29	30	18	0	24	0
	Pits	30	90	30	330	205	145	150	90	0	120	0
2004	Farms	0	0	0	6	3	0	3	1	0	2	0
	Fields	0	0	0	36	18	0	18	6	0	11	0
	Pits	0	0	0	180	90	0	90	30	0	55	0
2005	Farms	0	1	1	4	6	4	2	3	0	4	1
	Fields	0	6	6	30	36	30	12	18	0	24	6
	Pits	0	30	30	150	180	150	60	90	0	120	30
2006	Farms	1	1	1	7	4	3	5	7	0	4	1
	Fields	6	6	6	39	24	17	40	36	0	25	12
	Pits	30	30	30	195	120	85	200	180	0	125	60
2007	Farms	0	3	1	7	6	2	5	7	1	4	2
	Fields	0	18	6	42	36	12	36	36	6	24	12
	Pits	0	90	30	210	180	60	180	180	30	120	60
2008	Farms	0	6	1	6	6	2	6	7	1	4	3
	Fields	0	30	3	36	36	11	39	36	6	18	12
	Pits	0	150	15	180	180	55	195	180	30	90	60
2009	Farms	1	4	3	7	7	2	6	9	1	4	3
	Fields	6	24	18	40	42	12	42	52	6	19	12
	Pits	30	120	90	200	210	60	210	260	30	95	60
2010	Farms	2	5	2	9	4	2	8	8	0	1	3
	Fields	11	49	14	64	31	20	69	72	0	15	18
	Pits	55	245	70	320	155	100	345	360	0	75	90

Table 2. Soil texture measured in selected fields of sugarcane in the Midlands North region of the South African sugarcane industry.

Farm	Ecozone	Field	Sand	Silt	Clay	Organic matter
1	4 = reatively high absolute counts of larvae	10	54	8	38	3.2
		51	83	4	13	2.9
		85	57	7	36	3.4
		112	56	6	38	2.7
		162	51	9	40	3.8
		172	73	6	21	1.8
2		1	63	5	32	3.1
		33	62	6	32	3.0
		32	62	6	32	3.3
		N001	65	6	29	4.0
		N010	58	8	34	3.6
		45	61	5	34	2.9
1	8 = relatively low absolute counts of larvae	1	28	17	55	5.1
		6	26	15	59	3.8
		8	25	15	50	5.2
		9	30	17	53	4.9
		10	26	17	57	4.8
		11	27	15	53	5.0
2		C004	30	15	55	6.3
		C006	20	23	57	4.4
		C013	22	17	61	4.9
		D016C	22	15	63	3.8
		D018A	24	15	61	4.9
		D018B	21	12	67	3.4